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(54) Title: COMBINATION TREATMENTS WITH GASTRIN AGONISTS FOR DIABETES AND RELATED DISEASES

(57) Abstract: The invention relates to compositions, conjugates, and methods for the prevention and/or treatment of a condition and/or disease comprising a therapeutically effective amount of at least one gastrin agonist and at least one growth/hormone regulatory factor(s), and optionally at least one gastrin compound. The compositions, conjugates, and methods provide beneficial effects, in particular sustained beneficial effects, in the prevention and/or treatment of conditions and/or diseases including diabetes, hypertension, chronic heart failure, fluid retentive states, obesity, metabolic syndrome and related diseases and disorders. Combinations of at least one gastrin agonist and at least one growth/hormone regulatory factor(s), and optionally at least one gastrin compound can be selected to provide additive, complementary or synergistic effects.

Title: Combination Treatments with Gastrin Agonists for Diabetes and Related Diseases

FIELD OF THE INVENTION

The invention relates generally to compositions, conjugates, and methods comprising gastrin agonists and growth/hormone regulatory factors, and optionally gastrin compounds, and uses thereof.

BACKGROUND OF THE INVENTION

The insulin dependent diabetic population including both Type I and Type II diabetics, is estimated to be approximately 4 million people in the United States and approximately 7-8 million people worldwide. The population ranges from end stage insulin dependent diabetics (transplant, severe complications) to new onset insulin diabetics. Many pharmaceutical compositions and methods have been proposed to treat and/or cure diabetes. One approach is directed at enhancing islet neogenesis. Islet neogenesis is the process by which islets are formed from precursor stem cells in the ducts of the developing fetal pancreas. Methods for treating diabetes based on islet neogenesis have been described in US Patent Nos. 5,885,956, 6,288,301 and 6,558,952.

The current invention addresses the need for additional therapies for the treatment of diabetes and related diseases, disorders, and conditions.

SUMMARY OF THE INVENTION

Specifically disclosed herein is the co-administration of at least one gastrin agonist with at least one growth/hormone regulatory factor, and optionally at least one gastrin compound to prevent and/or treat diabetes and related diseases, disorders, or conditions as well as prevent and/or treat complications associated with diabetes, and related diseases, disorders, or conditions.

The therapeutic strategy of the present invention relates to the modification of multiple pathophysiological processes using innovative combinations of compounds that have distinct complementary, additive or synergistic mechanisms of action to provide safe and effective treatments for conditions and/or diseases disclosed herein. Compositions, conjugates, or methods, comprising gastrin agonists, growth/hormone regulatory factors, and optionally gastrin compounds employing different mechanisms to achieve maximum therapeutic efficacy, may improve tolerance to therapy with a reduced risk of side effects that may result from higher doses or longer term monotherapies (i.e., therapies with each compound alone). A composition, conjugate, or method of the invention may permit the use of lower doses of the compounds with reduced adverse toxic effects of each compound. A suboptimal dosage may provide an increased margin of safety, and may also reduce the cost of a drug necessary to achieve prophylaxis and therapy. In certain aspects of the invention, the increased convenience of a single combination dosage unit may result in enhanced compliance. Other advantages of a composition, conjugate, or combination therapy may include higher stability towards degradation and metabolism, longer duration of action, and/or longer duration of action or effectiveness at particularly low doses.

Broadly stated, the invention relates to compositions, conjugates, and methods for the prevention and/or treatment of a condition and/or disease disclosed herein comprising a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound that provide one or more beneficial effects.

In an aspect, the invention relates to compositions, conjugates, and methods for the prevention and/or treatment of a condition and/or disease disclosed herein comprising a therapeutically effective amount of at least

one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound that provide one or more beneficial effects.

In an aspect, the invention relates to compositions, conjugates, and methods for the prevention and/or treatment of diabetes in a subject receiving insulin comprising a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound that provide one or more beneficial effects.

A composition, conjugate, or method of the invention may provide sustained beneficial effects following treatment or termination of treatment. Prolonged efficacy may be evidenced by increased C-peptide production, increased in pancreatic insulin production, decreased insulin dependence, and/or about normal or reduced blood glucose levels relative to each compound alone.

In an aspect, the invention contemplates a composition, preferably a pharmaceutical composition, comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound. In another aspect the invention provides a pharmaceutical composition comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound that provide beneficial effects relative to each compound alone, preferably sustained beneficial effects, following treatment. A pharmaceutical composition may optionally comprise a pharmaceutically acceptable carrier, excipient, or vehicle.

In another aspect, the invention relates to a combination, such as a combined preparation or pharmaceutical composition, comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound and at least one additional pharmaceutically acceptable carrier, excipient, or vehicle.

The invention also contemplates a pharmaceutical composition in separate containers and intended for simultaneous or sequential administration to provide one or more beneficial effects, preferably sustained beneficial effects, comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, together with pharmaceutically acceptable carriers, excipients, or vehicles.

The invention further contemplates a conjugate comprising at least one gastrin agonist interacting with or linked to at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, in particular to provide one or more beneficial effects.

The invention still further contemplates methods for preparing compositions and conjugates of the invention that result in compositions and conjugates with one or more beneficial effects, preferably sustained beneficial effects.

In an aspect the invention relates to a medicinal combination of active principles, having, jointly, a complementary and/or synergistic action, this being for the treatment of diabetes, in particular of Type I diabetes.

In an aspect the invention relates to a medicinal combination of active principles, having, jointly, a complementary and/or synergistic action, this being for the treatment of diabetes, in particular of Type II diabetes.

In an aspect of the invention, a method is provided for preparing a pharmaceutical composition of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin

compound, in particular adapted to provide one or more beneficial effects following treatment, comprising preparing a composition comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, and a pharmaceutically acceptable carrier, excipient, or vehicle.

In another aspect of the invention, a method is provided for preparing a stable pharmaceutical composition comprising mixing at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, and a pharmaceutically acceptable carrier, excipient, or vehicle effective to physically stabilize the composition and in particular adapted to provide one or more beneficial effects, preferably sustained beneficial effects.

The invention relates to a combination treatment for preventing and/or treating a condition and/or disease disclosed herein in a subject comprising administering to the subject a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, in particular to provide one or more beneficial effects. In an aspect of the invention a combination treatment is provided for preventing and/or treating a condition and/or disease disclosed herein in a subject comprising administering to the subject a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, in particular to provide one or more beneficial effects. In an aspect the invention provides a combination treatment or intervention which provides one or more sustained beneficial effects following treatment.

The invention further relates to the use of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, a composition, or conjugate of the invention for preventing, delaying progression of, and/or ameliorating disease severity, disease symptoms, and/or periodicity of recurrence of a condition and/or disease disclosed herein. In particular, the invention relates to the prevention, delay of progression, and/or treatment, in a subject, of conditions and/or diseases using at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, a composition, or conjugate of the invention.

In an aspect, the invention provides a method for the prevention and/or intervention of a condition and/or disease disclosed herein in a subject comprising administration of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention. A gastrin agonist, growth/hormone regulatory factor, and optionally gastrin compound, or a composition or conjugate may be directly administered to a subject or contacted with cells (e.g. stem cells or progenitor cells) and administered to a subject.

In other aspects, the invention provides a method for the prevention and/or intervention of a condition and/or disease disclosed herein in a subject comprising administration of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound to a subject in need thereof to provide beneficial effects.

The invention provides in some aspects methods for the potentiation of a gastrin agonist in the treatment of a condition and/or disease in a subject, in particular diabetes and related diseases, disorders, or conditions, comprising co-administering at least one growth/hormone regulatory factor and optionally at least one gastrin compound to the subject.

The invention provides in some aspects methods for the potentiation of a growth/hormone regulatory factor in the treatment of a condition and/or disease in a subject, in particular diabetes and related diseases, disorders, or conditions, comprising co-administering at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound to the subject.

In another aspect, the invention provides a method for the prevention and/or intervention of a condition and/or disease disclosed herein in a subject comprising co-administering at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound to a subject in need thereof.

In a particular aspect, the invention relates to inducing islet neogenesis in a subject comprising contacting islet precursor cells with at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition, or conjugate of the invention in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

In another aspect, the invention relates to a method for treating diabetes mellitus in a patient in need thereof by administering at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound or a composition comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound in amounts sufficient to effect differentiation of the patient's pancreatic islet precursor cells to mature insulin-secreting cells and/or to stimulate insulin synthesis in existing islet cells.

The invention provides methods for treating cells using at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or compositions, or conjugates of the invention. In particular, the invention relates to a method for expanding and differentiating stem cells or progenitor cells into insulin secreting cells, enhancing proliferation of insulin secreting cells, and/or sustaining islet cells or precursor cells. Cells may be contacted with a gastrin agonist(s), a growth/hormone regulatory factor(s), and optionally a gastrin compound(s) in culture or in a subject.

In an aspect, a method is provided for treating a condition and/or disease comprising administering at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention with a plurality of cells to a subject in need thereof to thereby produce one or more beneficial effects, preferably sustained beneficial effects. In an embodiment, the compounds, composition, or conjugate are administered systemically.

In another aspect, the invention provides a method for treating a subject with a condition and/or disease disclosed herein comprising contacting *ex vivo* a plurality of cells with at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention, optionally culturing the cells, and administering the cells to the subject in need thereof.

Also provided in particular aspects of the invention are methods and compositions for treating diabetes in a patient in need thereof by implanting into a diabetic patient pancreatic islet cells that have been exposed in culture to a sufficient amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention, to increase the number of pancreatic beta cells in the islets; optionally the population of pancreatic beta cells can be grown in culture for a time sufficient to expand the population of β-cells prior to transplantation.

In aspects of the invention, a therapeutically effective amount of an insulin or insulin analog is also administered, separately or together with a gastrin agonist(s), a growth/hormone regulatory factor(s), and optionally a gastrin compound(s), to the subject.

In aspects of the invention, a therapeutically effective amount of an immunosuppressive agent is also administered, separately or together with a gastrin agonist(s), growth/hormone regulatory factor(s), and optionally gastrin compound(s), to the subject.

In aspects of the invention, a therapeutically effective amount of an insulin sensitivity enhancer is also administered, separately or together with a gastrin agonist(s), growth/hormone regulatory factor(s), and optionally gastrin compound(s), to the subject.

In aspects of the invention, a therapeutically effective amount of glucose lowering agent is also administered, separately or together with a gastrin agonist(s), growth/hormone regulatory factor(s), and optionally gastrin compound(s), to the subject.

In aspects of the invention, a therapeutically effective amount of an insulin secretagogue is also administered, separately or together with a gastrin agonist(s), growth/hormone regulatory factor(s), and optionally gastrin compound(s), to the subject.

In aspects of the invention, a therapeutically effective amount of an antiobesity or appetite regulating agent is also administered, separately or together with a gastrin agonist(s), growth/hormone regulatory factor(s), and optionally gastrin compound(s), to the subject.

The invention also contemplates the use of a composition comprising a combination of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound for the preparation of one or more medicament for preventing and/or treating a condition and/or disease. The invention further contemplates use of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound for the manufacture of a medicament for the treatment of a condition and/or disease. Still further the invention provides use of a gastrin agonist for the manufacture of a medicament for the treatment of a condition and/or disease to be used in combination with a growth/hormone regulatory factor and optionally a gastrin compound.

In an aspect, the invention relates to the use of synergistically effective amounts of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound for the preparation of a medicament for preventing or treating a condition and/or disease. In another aspect, the invention relates to the use of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound for the preparation of a medicament which has a protracted profile of action relative to each compound alone or any two of the compounds. The invention additionally provides uses of a pharmaceutical composition and a conjugate of the invention in the preparation of medicaments for the prevention and/or treatment of conditions and/or diseases. The medicaments may provide beneficial effects, preferably sustained beneficial effects following treatment.

Since the present invention relates to a method of prevention and/or treatment comprising a combination of active agents which may be administered separately or as conjugates, the invention also provides a kit comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, and a pharmaceutical composition, or conjugate of the invention in kit form.

In aspects of the compositions, conjugates, methods, uses and kits comprise or consist of at least one growth/hormone regulatory factor and at least one gastrin compound. In other aspects of the compositions, conjugates, methods, uses and kits comprise or consist of at least one growth/hormone regulatory factor, at least one gastrin agonist and at least one gastrin compound.

These and other aspects, features, and advantages of the present invention should be apparent to those skilled in the art from the following detailed description.

Glossary

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, the terms "comprising," "including," and "such as" are used in their open and non-limiting sense.

The recitation of numerical ranges by endpoints herein includes all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term "about." The term "about" means plus or minus 0.1 to 50%, 5-50%, or 10-40%, preferably 10-20%, more preferably 10% or 15%, of the number to which reference is being made.

Further, it is to be understood that "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to "a compound" includes a mixture of two or more compounds. Thus, the phrase "a gastrin compound" as used herein can also mean "one or more gastrin compound" or "at least one gastrin compound". The phrase "a gastrin agonist", as used herein can also mean "one or more gastrin agonist" or "at least one gastrin agonist". The phrase "a growth/hormone regulatory factor", as used herein can also mean "one or more growth/hormone regulatory factor" or "at least one growth/hormone regulatory factor".

Selected compounds described herein contain one or more asymmetric centers and may give rise to enantiomers, diasteriomers, and other stereoisomeric forms which may be defined in terms of absolute stereochemistry as (R)- or (S)-. Therefore, the invention includes all such possible diasteriomers and enantiomers as well as their racemic and optically pure forms. Optically active (R)- and (S)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and A geometric isomers. All tautomeric forms are intended to be included within the scope of the invention.

The terms "administering" and "administration" refer to the process by which a therapeutically effective amount of compounds or a composition or conjugate contemplated herein is delivered to a subject for prevention and/or treatment purposes. Compositions are administered in accordance with good medical practices taking into account the subject's clinical condition, the site and method of administration, dosage, patient age, sex, body weight, and other factors known to physicians.

The terms "subject", "individual" or "patient" refer to an animal including a warm-blooded animal such as a mammal, which is afflicted with or suspected of having or being pre-disposed to a condition and/or disease as disclosed herein. Preferably, the terms refer to a human. The terms also include domestic animals bred for food, sport, or as pets, including horses, cows, sheep, poultry, fish, pigs, cats, dogs, and zoo animals. The

methods herein for use on subjects/individuals/patients contemplate prophylactic as well as curative use. Typical subjects for treatment include persons susceptible to, suffering from or that have suffered a condition and/or disease disclosed herein.

The term "pharmaceutically acceptable carrier, excipient, or vehicle" refers to a medium which does not interfere with the effectiveness or activity of an active ingredient and which is not toxic to the hosts to which it is administered. A carrier, excipient, or vehicle includes diluents, binders, adhesives, lubricants, disintegrates, bulking agents, wetting or emulsifying agents, pH buffering agents, and miscellaneous materials such as absorbants that may be needed in order to prepare a particular composition. The use of such media and agents for an active substance is well known in the art. In certain aspects of the invention, a carrier, excipient, or vehicle is selected to stabilize a gastrin compound, a growth/hormone regulatory factor, and a gastrin agonist.

"Pharmaceutically acceptable salt(s)," means a salt that is pharmaceutically acceptable and has the desired pharmacological properties. By pharmaceutically acceptable salts is meant those salts which are suitable for use in contact with the tissues of a subject or patient without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are described for example, in S. M. Berge, et al., J. Pharmaceutical Sciences, 1977, 66:1. Suitable salts include salts that may be formed where acidic protons in the compounds are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with alkali metals, e.g. sodium and potassium, magnesium, calcium, and aluminum. Suitable organic salts include those formed with organic bases such as the amine bases, e.g. ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Suitable salts also include acid addition salts formed with inorganic acids (e.g. hydrochloride and hydrobromic acids) and organic acids (e.g. acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic acids such as methanesulfonic acid and benezenesulfonic acid). When there are two acidic groups present, a pharmaceutically acceptable salt may be a mono-acid-mono-salt or a di-salt; and similarly where there are more than two acidic groups present, some or all of such groups can be salified.

The terms "preventing and/or treating", "prevention and/or treatment", or "prevention and/or intervention" refer to the administration to a subject of biologically active agents either before or after onset of a condition and/or disease. A treatment may be either performed in an acute or chronic way. In particular, prevention includes the management and care of a subject at risk of developing a condition and/or disease disclosed herein prior to the clinical onset of the condition and/or disease. Treatment or intervention refers to the management and care of a subject at diagnosis or later. An objective of prevention, treatment, or intervention is to combat the condition and/or disease and includes administration of the active compounds to prevent or delay the onset of the symptoms or complications, or alleviating the symptoms or complications, or eliminating or partially eliminating the condition and/or disease.

A "beneficial effect" refers to an effect of a combination of one or more gastrin agonist, one or more growth/hormone regulatory factor, and optionally one or more gastrin compound, or composition or conjugate thereof that is greater than the effect of the compounds alone. The beneficial effect includes favorable pharmacological and/or therapeutic effects, and improved pharmacokinetic properties and biological activity. A beneficial effect may be an additive effect, complementary or synergistic effect. In aspects of the invention, beneficial effects include but are not limited to the following: reduced or absent islet inflammation, decreased

disease progression, increased survival, or elimination or partial elimination of a condition and/or disease. In particular aspects, the beneficial effect is a "sustained beneficial effect" where the beneficial effect is sustained for a prolonged period of time after termination of treatment. In an embodiment, one or more of the aforementioned effects are sustained for a prolonged period of time after termination of treatment. A beneficial effect may be sustained for at least about 2, 4, 6, 8, 10, 2 to 4 weeks, 2 to 6 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment. The period of time a beneficial effect is sustained may correlate with the duration and timing of the treatment. A subject may be treated continuously for about 2 to 8 weeks, 2 to 12 weeks, 2 to 16 weeks, 2 weeks to 6 months, 2 weeks to 12 months, or periodically. A sustained beneficial effect may manifest as one or more of increased C-peptide production, increased pancreatic insulin production, about normal or reduced blood glucose levels for a prolonged period following treatment, decreased insulin dependence or delivery, increased beta cell production and/or inhibition of programmed cell death (apoptosis), or reduction in insulin use in a subject.

The beneficial effect may be a statistically significant effect in terms of statistical analysis of an effect of the compounds versus the effects of each of the compounds or two compounds alone. "Statistically significant" or "significantly different" effects or levels with the compounds compared with each compound or two compounds alone may represent levels that are higher or lower than a standard. In embodiments of the invention, the difference may be 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40 or 50 times higher or lower compared with the effect obtained with each compound or two compounds alone.

An "additive effect" of one or more gastrin agonist, one or more growth/hormone regulatory factor, and optionally one or more gastrin compound refers to an effect that is equal to the sum of the effects of the three individual compounds

A "synergistic effect" of one or more gastrin agonist, one or more growth/hormone regulatory factor, and optionally one or more gastrin compound refers to an effect that is greater than the additive effect which results from the sum of the effects of the individual compounds.

"Combination treatment", "combination therapy", "co-administration" and "administering in combination" are used interchangeably herein and mean that the active ingredients are administered concurrently to a patient being treated. When administered in combination each component may be administered at the same time, or sequentially in any order at different points in time. Therefore, each component may be administered separately, but sufficiently close in time to provide the desired effect, in particular a beneficial, complementary, additive, or synergistic effect. The first compound may be administered in a regimen which additionally comprises treatment with a second and third compound. In certain embodiments, the term refers to administration of one or more gastrin agonist, one or more growth/hormone regulatory factor, and optionally one or more gastrin compound to a patient within one year, including separate administration of three medicaments each containing one of the compounds as well as simultaneous administration whether or not the compounds are combined in one formulation or whether they are three separate formulations.

A "medicament" refers to a pharmaceutical composition suitable for administration of a pharmaceutically active compound(s) (e.g. one or more gastrin agonist, one or more growth/hormone regulatory factor, and optionally one or more gastrin compound) to a patient.

"Therapeutically effective amount" relates to the amount or dose of active compounds (e.g. gastrin agonist, growth/hormone regulatory factor, and optionally gastrin compound), compositions or conjugates of the invention that will lead to one or more desired beneficial effects, preferably one or more sustained beneficial effects. A "therapeutically effective amount" can provide a dosage which is sufficient in order for prevention and/or treatment of a condition and/or disease in a subject to be effective compared with no treatment.

"Synergistically effective amount" relates to the amount of dose of active compounds (e.g. gastrin agonist, growth/hormone regulatory factor, and optionally gastrin compound), compositions or conjugates of the invention that will provide a synergistic effect, in particular a synergistic beneficial effect.

The expression "complementary action" or "complementary effect" refers to the pharmacological action of one, two, three or more different compounds making it possible to act on the same pathology via different pharmacological mechanisms, for example the combined use of a gastrin agonist, growth/hormone regulatory factor, and optionally gastrin compound.

The term "potentiation" refers to an increase of a corresponding pharmacological activity or therapeutic effect. Potentiation of one component of a combination or composition of the present invention by co-administration of the other components according to the present invention means that an effect is being achieved that is greater than that achieved with one component alone.

"Suboptimal dose" or suboptimal dosage" refers to a dose or dosage of one or more active compound which is less than the optimal dose or dosage for that compound when used in monotherapy.

The terms "associated", "linked", "interact", "interaction", or "interacting" refer to any physical association between molecules. The terms preferably refer to a stable association between molecules due to, for example, electrostatic, hydrophobic, ionic, hydrogen-bond interactions, or covalent interactions.

"Sequence identity"of two amino acid sequences, or of two nucleic acid sequences is defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues in a polypeptide or nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining amino acid or nucleic acid sequence identity can be achieved in various conventional ways, for instance, using publicly available computer software including the GCG program package (Devereux J. et al., Nucleic Acids Research 12(1): 387, 1984); BLASTP, BLASTN, and FASTA (Atschul, S.F. et al. J. Molec. Biol. 215: 403-410, 1990). The BLAST programs are publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al. NCBI NLM NIH Bethesda, Md. 20894; Altschul, S. et al. J. Mol. Biol. 215: 403-410, 1990). Skilled artisans can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Methods to determine identity and similarity are codified in publicly available computer programs.

An "analog" refers to a polypeptide wherein one or more amino acid residues of a parent or wild-type polypeptide have been substituted by another amino acid residue, one or more amino acid residues of a parent or wild-type polypeptide have been inverted, one or more amino acid residues of the parent or wild-type polypeptide have been deleted, and/or one or more amino acid residues have been added to the parent or wild-type polypeptide. Such an addition, substitution, deletion, and/or inversion may be at either of the N-terminal or

C-terminal end or within the parent or wild-type polypeptide, or a combination thereof. Typically "an analog" is a peptide wherein 6 or less amino acids have been substituted and/or added and/or deleted from the parent or wild-type peptide, more preferably a peptide wherein 3 or less amino acids have been substituted and/or added and/or deleted from the parent or wild-type polypeptide, and most preferably, a peptide wherein one amino acid has been substituted and/or added and/or deleted from the parent or wild-type polypeptide.

Mutations may be introduced into a polypeptide by standard methods, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative substitutions can be made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which an amino acid residue is replaced with an amino acid residue with a similar side chain. Amino acids with similar side chains are known in the art and include amino acids with basic side chains (e.g. Lys, Arg, His), acidic side chains (e.g. Asp, Glu), uncharged polar side chains (e.g. Gly, Asp, Glu, Ser, Thr, Tyr and Cys), nonpolar side chains (e.g. Ala, Val, Leu, Iso, Pro, Trp), beta-branched side chains (e.g. Thr, Val, Iso), and aromatic side chains (e.g. Tyr, Phe, Trp, His). Mutations can also be introduced randomly along part or all of the native sequence, for example, by saturation mutagenesis. Following mutagenesis the variant polypeptide can be recombinantly expressed.

A "derivative" refers to a polypeptide in which one or more of the amino acid residues of a parent polypeptide have been chemically modified. Derivatives may be obtained by chemically modifying one or more amino acid residues of the parent polypeptide or analog thereof, for instance by alkylation, acylation, glycosylation, pegylation, ester formation, deamidation, amide formation, or by introducing a lipophilic functionality. In aspects of the invention, "a derivative" designates a peptide or analogue thereof which is chemically modified by introducing an ester, alkyl or lipophilic functionality on one or more amino acid residues of the peptide or analogue thereof.

"Bioavailability" refers to the extent to which a drug or metabolite is absorbed into the general circulation of a subject and becomes available at the site of action of the drug in the subject.

A "chimeric polypeptide" comprises all or part (preferably biologically active) of a selected polypeptide operably linked to a heterologous polypeptide (i.e., a polypeptide other than the selected polypeptide). Within the fusion protein, the term "operably linked" is intended to indicate that a selected polypeptide and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the N-terminus or C-terminus of a selected polypeptide. Chimeric and fusion proteins can be produced by standard recombinant DNA techniques.

A "gastrin agonist" refers to any substance that fully or partially mimics a reaction, activity, or function of a gastrin compound or initiates such reaction, activity, or function, or reduces or prevents inhibition of any reaction, activity or function of a gastrin compound. In aspects of the invention, a gastrin agonist is a gastrin secretagogue. In aspects of the invention, a gastrin agonist is selected that provides, in combination with a growth/hormone regulatory factor, and optionally a gastrin compound, therapeutically effective amounts of gastrin in a subject (e.g., a diabetic subject). In particular aspects of the invention a gastrin agonist is selected that provides an about 1.5 to 1000 fold, 5 to 1000 fold, 10 to 1000 fold, 10 to 500 fold, 10 to 100 fold, 5 to 100, 10 to 50, 5 to 50, 10 to 25, 1.5 to 10, 1.5 to 5, 1.5 to 3, 1.5 to 5, 1.5 to 10, 1.5 to 20, 1.5 to 25, 3 to 5, 3 to 10, 3 to 15, 3 to 25, 5 to 15, or 5 to 20 fold increase in plasma gastrin. The term includes analogs, derivatives, fragments and modifications of a wild-type gastrin agonist and chimeric polypeptides comprising a gastrin agonist. Gastrin

agonists and gastrin compounds are mutually exclusive and differ where both are used in the compositions, conjugates and methods of the invention. Generally, a gastrin agonist is a proton pump inhibitor or a histamine-2 receptor antagonist.

A "proton pump inhibitor" and "PPI" are used interchangeably herein and include a substance which inhibits gastric acid secretion by blocking the proton pump and/or increasing gastrin secretion.

In particular, the term "proton pump inhibitor" refers to any acid labile pharmaceutical agent possessing pharmacological activity as an inhibitor of H^+/K^+-ATPase. More particularly it contemplates substances which covalently bind to H+/K+-ATPase, the enzyme responsible for gastric acid secretion. [See the following publications relating to PPIs: Fellenius et al., Substituted Benzimidazoles Inhibit Gastric Acid Secretion by Blocking H^+, K^+-ATPase, Nature, 290:159-161 (1981); Wallmark et al, The Relationship Between Gastric Acid Secretion and Gastric H^+, K^+-ATPase Activity, J. Biol.Chem., 260:13681-13684 (1985); Fryklund et al., Function and Structure of Parietal Cells After H^+, K^+-ATPase Blockade, Am. J. Physiol., 254 (3 PT 1); G399-407 (1988)]. A proton pump inhibitor may be selected for use in the compositions, conjugates, methods and uses disclosed herein based on one or more of the following properties: (i) a bioavailability greater than about 40%, 41%, 42%, 43%, 44%, 45%, 50%, 50% to 55%, 60%, 65%, 70%, 75%, 66%, 77%, 78%, 79%, 80%, 80% to 85%, 90%, 95%, 100%, 65-100%, or 80-95%; (ii) a plasma elimination half life greater than about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, or 3 hours, and (iii) it does not bind to cysteine 892 of the alpha subunit of the proton pump. Generally, omeprazole, its salts, polymorphs, and any of its analogs and derivatives thereof are not encompassed within the term "proton pump inhibitor".

In aspects of the invention, a PPI includes compounds comprising a 2-[(2-pyridinyl) methylsulphinyl]-1H-benzimidazole skeleton or a related skeleton, which may optionally be substituted in various forms. A proton pump inhibitor may, if desired, be in the form of free base, free acid, salt, ester, solvates (in particular hydrates), anhydrate, amide, enantiomer, isomer, tautomer, prodrug, polymorph, derivative, or the like, provided that the free base, salt, ester, hydrate, amide, enantiomer, isomer, tautomer, prodrug, or any other pharmacologically suitable derivative is therapeutically active.

The following PPIs may be mentioned in the context of the present invention: 2-[2-(N-isobutyl-N-methylamino)benzyl-sulphinyl]benzimidazole (INN: leminoprazole) (DE-A-3531487); 2-(4-methoxy-6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-ylsulphinyl)-1H-benzimidazole (INN: nepaprazole) (EP-A-0 434 999); 2-(4-methoxy-3-methyl-pyridin-2-ylmethylsulphinyl)5-pyrrol-1-y-1H-benzimidazole (IY-81149), 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methylsulphinyl]-1-H-inidazo[4,5-b]pyridine(tenatoprazole), 2-[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl)methylsulphinyl]-1H-benzimidazole (INN: lansoprazole) (EP-A 0 174 726); and 2-{[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]-methylsulphinyl}-1H-benzimidazole (INN: rabeprazole) (EP-A 0 184 322, EP-A 0 254 588, EP-A-0 261 478, EP-A-0 268 956); and in particular 5-difluoromethoxy-2-[(3,4-dimethoxy-2-pyridinyl)methylsulphinyl]-1H-benzimidazole (INN: pantoprazole) (EP-A-0 124 495, EP-A-0 166 287); and (−)-5-difluoromethoxy-2-[(3,4-dimethoxy-2-pyridinyl)methylsulphinyl]-1H-benzimidazole [(−)pantoprazole].

Other proton pump inhibitors include but are not limited to: soraprazan (Altana); ilaprazole (U.S. Pat. No. 5,703,097) (Il-Yang); AZD-0865 (AstraZeneca); dontoprazole; habeprazole; perprazole; ransoprazole; pariprazole; YH-1885 (PCT Publication WO 96/05177) (SB-641257) (2-pyrimidinamine, 4-(3,4-dihydro-1-

methyl-2(1H)-isoquinolinyl)-N-(4-fluorophenyl)-5,6-dimethyl-, monohydrochloride) (YuHan); phenylalkyl-amino derivatives of condensed carbapenem cpds (WO-A-9523149); BY-112 (Altana); SPI-447 (Imidazo(1,2-a)thieno(3,2-c)pyridin-3-amine,5-methyl-2-(2-methyl-3-thienyl) (Shinnippon); 3-hydroxymethyl-2-methyl-9-phenyl-7H-8,9-dihydro-pyrano(2,3-c)-imidazo(1,2-a)pyridine (PCT Publication WO 95/27714) (AstraZeneca); Pharmaprojects No. 4950 (3-hydroxymethyl-2-methyl-9-phenyl-7H-8,9-dihydro-pyrano(2,3-c)-imidazo(1,2-a)pyridine) (AstraZeneca, ceased) WO 95/27714; Pharmaprojects No. 4891 (EP 700899) (Aventis); Pharmaprojects No. 4697 (PCT Publication WO 95/32959) (AstraZeneca); H-335/25 (AstraZeneca); T-330 (Saitama 335) (Pharmacological Research Lab); Pharmaprojects No. 3177 (Roche); BY-574 (Altana); Pharmaprojects No. 2870 (Pfizer); AU-1421 (EP 264883) (Merck); AU-2064 (Merck); AY-28200 (Wyeth); Pharmaprojects No. 2126 (Aventis); WY-26769 (Wyeth); pumaprazole (PCT Publication WO 96/05199) (Altana); YH-1238 (YuHan); Pharmaprojects No. 5648 (PCT Publication WO 97/32854) (Dainippon); BY-686 (Altana); YM-020 (Yamanouchi); GYKI-34655 (Ivax); FPL-65372 (Aventis); Pharmaprojects No. 3264 (EP 509974) (AstraZeneca); nepaprazole (Toa Eiyo); HN-11203 (Nycomed Pharma); OPC-22575; pumilacidin A (BMS); saviprazole (EP 234485) (Aventis); SKandF-95601 (GSK, discontinued); Pharmaprojects No. 2522 (EP 204215) (Pfizer); S-3337 (Aventis); RS-13232A (Roche); AU-1363 (Merck); SKand F-96067 (EP 259174) (Altana); SUN 8176 (Daiichi Phama); Ro-18-5362 (Roche); ufiprazole (EP 74341) (AstraZeneca); Bay-p-1455 (Bayer); BY308; perprazole; [4-(2,2,2-trifluoroethoxy)-3-methyl-2-pyridyl]-methyl]sulfenamide; (Z)-5-methyl-2-[2-(1-naphthyl)ethenyl]-4-piperidinopyridine HCl; 2-(4-cyclohexyloxy-5-methyl pyridin-2-yl)-3-(1-naphthyl)-1-propanol; methyl 2-cyano-3-(ethylthio)-3-(methylthio)-2propenoate; 2-((4-methoxy-2-pyridyl)methylsulphinyl)-5-(1,1,2,2-tetrafluoroethoxy)-1H-benzimidazole sodium; 2-[[[4-(2,2,3,3,4,4,4-heptafluoro butoxy)-2-pyridyl]methyl)sulfinyl]-1H-thieno[3,4-d]imidazole; 2-[[[4-(2,2,2-trifluoro ethoxy)-3-methyl-2-pyridyl]methyl] sulfinyl]-1H-benzimidazole; 2-[[[4-(2,2,2-trifluoro ethoxy)-3-methyl-2-pyridyl]methyl] sulfinyl]-1H-benzimidazole; 2-methyl-8-(phenyl methoxy)-imidazo(1,2-A)-pyridine-3-acetonitrile; (2-((2-dimethylaminobenzyl)sulfinyl)-benzimidazole); 4-(N-allyl-N-methyl amino)-1-ethyl-8-((5-fluoro-6-methoxy-2-benzi midazolyl)sulfinylmethyl)-1-ethyl 1,2,3,4-tetrahydroquinolone; 2-[[(2-dimethylamino phenyl)methyl]sulfinyl]-4,7-dimethoxy-1H-benzimidazole; 2-[(2-(2-pyridyl)phenyl) sulfinyl)-1H-benzimidazole; (2-[(2-amino-4-methylbenzyl)sulfinyl]-5-methoxy benzo[d]imidazole; (4(2-methylpyrrol-3-yl)-2-guanid isothiazole); 4-(4-(3-(imidazole) propoxy)phenyl)-2phenylthiazole; (E)-2-(2-(4-(3-(dipropylamino)butoxy)phenyl)-ethenyl)benzoxazole; (E)-2-(2-(4-(3-(dipropylamino) propoxy)phenyl)ethenyl)-benzothiazole; Benzeneamine, 2-[[(5-methoxy-1H-benzimidazol-2-yl)sulfinyl]methyl)-4-methyl-; 2,3-dihydro-2-methoxycarbonylamino-1,2-benzisothiazol-3-one; 2-(2-ethyl aminophenylmethylsulfinyl)-5,6-dimethoxybenzimidazole; 2-methyl-8-(phenyl methoxy)imidazo[1,2-a)pyridine-3-acetonitrile; 3-amino-2-methyl-8-phenyl methoxy imidazo[1,2-a)-pyrazine HCl; 2-[[(3-chloro-4-morpholino-2-pyridyl)methyl]-sulfinyl)-5-methoxy-(1H)-benzinidazole; [3-butyryl-4-(2-methylphenylamino)-8-methoxy-quinoline); 2-indanyl 2-(2-pyridyl)-2-thiocarbamoylacetate HCl; 2,3-dihydro-2-(2-pyridinyl)-thiazolo (3,2-a)-benzimidazole; 3-cyanomethyl-2-methyl-8-(3-methyl-2-butenyloxy)-(1,2-a)imidazo pyridine; zinc L-carnosine; or, a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, prodrug, or derivative of these compounds.

In certain aspects the proton pump inhibitor is selected from the group consisting of 2-[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl) methylsulphinyl]-1H-benzimidizole (lansoprazole), 2-{[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]me thylsulphinyl}-1H-benzimidazole(rabeprazole), 5-difluoromethoxy-2-[(3,4-di-methoxy-2-pyridinyl)-methylsulphinyl]-1H-benzimidazole (pantoprazole) and the hydrates, solvates, salts, hydrates of the salts and solvates of the salts thereof.

In certain aspects the proton pump inhibitor is selected from the group consisting of 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methylsulphinyl]-1H-benzimidazole (omeprazole), 5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulphinyl]-1H-benzimidazole(esomeprazole), 2-[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl) methylsulphinyl]-1H-benzimidizole(lansoprazole), 2-{[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulphinyl}-1H-benzimidazole(rabeprazole), 5-difluoromethoxy- 2-[(3,4-di-methoxy-2-pyridinyl)-methylsulphinyl]-1H-benzimidazole (pantoprazole) and the hydrates, solvates, salts, hydrates of the salts and solvates of the salts thereof.

Still other proton pump inhibitors contemplated by the present invention include those described in the following U.S. patent Nos: U.S. Pat. Nos. 4,628,098; 4,689,333; 4,786,505; 4,853,230; 4,965,269; 5,021,433; 5,026,560; 5,045,321; 5,093,132; 5,430,042; 5,433,959; 5,576,025; 5,639,478; 5,703,110; 5,705,517; 5,708,017; 5,731,006; 5,824,339; 5,855,914; 5,879,708; 5,948,773; 6,017,560; 6,123,962; 6,187,340; 6,296,875; 6,319,904; 6,328,994; 4,255,431; 4,508,905; 4,636,499; 4,738,974; 5,690,960; 5,714,504; 5,753,265; 5,817,338; 6,093,734; 6,013,281; 6,136,344; 6,183,776; 6,328,994; 6,479,075; 6,559,167.

In aspects of the invention a proton pump inhibitor is in the form of a salt. A salt of a proton pump inhibitor may be prepared from formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, ftimaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, stearic, salicylic, p-hydroxybenzoic, phenylacetic, mandelic, embonic, methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, toluenesulfonic, 2-hydroxyethanesulfonic, sulfanilic, cyclohexylaminosulfonic, algenic, β-hydroxybutyric, galactaric and galacturonic acids.

In an embodiment, acid addition salts are prepared from the free base of a proton pump inhibitor using conventional methods involving reaction of the free base with a suitable acid. Suitable acids for preparing acid addition salts include without limitation organic acids, such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like, as well as inorganic acids, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like.

In another embodiment, an acid addition salt is converted to a free base by treatment with a suitable base. In a further embodiment, an acid addition salt is a halide salt, which is prepared using hydrochloric or hydrobromic acids. In still another embodiment, the basic salt is an alkali metal salt, such as a sodium salt or copper salt.

Examples of salts of proton pump inhibitors include without limitation: a sodium salt form such as esomeprazole sodium, omeprazole sodium, rabeprazole sodium, pantoprazole sodium; or a magnesium salt form such as esomeprazole magnesium or omeprazole magnesium described in U.S. Pat. No. 5,900,424; a calcium salt form; or a potassium salt form such as the potassium salt of esomeprazole described in U.S. Pat. No. 6,511,996.

Other salts of esomeprazole are described in U.S. Pat. Nos. 4,738,974 and 6,369,085. Salt forms of pantoprazole and lansoprazole are disclosed in U.S. Pat. Nos. 4,758,579 and 4,628,098, respectively.

In an embodiment, esters of proton pump inhibitors are utilized. An ester may be prepared by functionalization of hydroxyl and/or carboxyl groups which may be present within the molecular structure of the drug. In another embodiment, the esters are acyl-substituted derivatives of free alcohol groups, such as moieties derived from carboxylic acids of the formula -RCOOR^1 where R^1 is an alkyl group in particular a lower alkyl group. An ester can be converted to a free acid, if desired, by using conventional procedures such as hydrogenolysis or hydrolysis.

A proton pump inhibitor or its salts can be in a crystalline form. Crystals of a proton pump inhibitor may contain variable amounts of solvent. Therefore, the term "proton pump inhibitor" includes all solvates, in particular all hydrates, of the proton pump inhibitors and their salts. In particular aspects of the invention the proton pump inhibitor is a salt or hydrate including without limitation pantoprazole-sodium sesquihydrate [pantoprazole-sodium×1.5 H_2O], (−)-pantoprazole-sodium sesquihydrate, pantoprazole-magnesium dihydrate, omeprazole-magnesium, omeprazole-magnesium tetrahydrate, esomeprazole-magnesium and esomeprazole-magnesium tetrahydrate

In various aspects of the invention, the proton pump inhibitor is a substituted bicyclic aryl-imidazole, wherein the aryl group may be, for example, a pyridine, a phenyl, or a pyrimidine group which is attached to the 4- and 5-positions of the imidazole ring. Proton pump inhibitors comprising a substituted bicyclic aryl-imidazole include, but are not limited to, omeprazole, hydroxyomeprazole, esomeprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, perprazole, tenatoprazole, ransoprazole, pariprazole, leminoprazole, or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, prodrug, or derivative thereof. (See, e.g., *The Merck Index,* Merck & Co. Rahway, N.J. (2001)).

Substituted bicyclic aryl-imidazole compounds as well as their salts, hydrates, esters, amides, enantiomers, isomers, tautomers, polymorphs, prodrugs, and derivatives may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry. See, e.g., *March, Advanced Organic Chemistry: Reactions, Mechanisms and Structure,* 4th Ed. (New York: Wiley-Interscience, 1992); Leonard et al., *Advanced Practical Organic Chemistry,* (1992); Howarth et al.; *Core Organic Chemistry* (1998); and Weisermel et al., *Industrial Organic Chemistry* (2002).

A tautomer of a substituted bicyclic aryl-imidazole includes without limitation tautomers of omeprazole such as those disclosed in U.S. Pat. Nos. 6,262,085; 6,262,086; 6,268,385; 6,312,723; 6,316,020; 6,326,384; 6,369,087; and 6,444,689; and U.S. Publication No. 02/0156103. An example of an isomer of a substituted bicyclic aryl-imidazole is an isomer of omeprazole including but not limited to an isomer disclosed in: Oishi et al., Acta Cryst. (1989), C45, 1921-1923; U.S. Pat. No. 6,150,380; U.S. patent publication No. 02/0156284; and PCT Publication No. WO 02/085889.

An amide of a bicyclic aryl-imidazole compound may be prepared using techniques known to those skilled in the art or described in the pertinent literature. For example, an amide may be prepared from esters, using suitable amine reactants, or they may be prepared from an anhydride or an acid chloride by reaction with an amine group e.g., ammonia or a lower alkyl amine.

Suitable polymorphs include but are not limited to the polymorphs described in PCT Publication No. WO 92/08716, and U.S. Pat. Nos. 4,045,563; 4,182,766; 4,508,905; 4,628,098; 4,636,499; 4,689,333; 4,758,579; 4,783,974; 4,786,505; 4,808,596; 4,853,230; 5,026,560; 5,013,743; 5,035,899; 5,045,321; 5,045,552; 5,093,132; 5,093,342; 5,433,959; 5,464,632; 5,536,735; 5,576,025; 5,599,794; 5,629,305; 5,639,478; 5,690,960; 5,703,110; 5,705,517; 5,714,504; 5; 5,731,006; 5,879,708; 5,900,424; 5,948,773; 5,948,789; 5,997,903; 6,017,560; 6,123,962; 6,147,103; 6,150,380; 6,166,213; 6,191,148; 5,187,340; 6,268,385; 6,262,086; 6,262,085; 6,296,875; 6,316,020; 6,328,994; 6,326,384; 6,369,085; 6,369,087; 6,380,234; 6,384,059, 6,428,810; 6,444,689; 6,462,058; 6,903,122; 6,933,389; and 6,939,971.

In aspects of the invention, a proton pump inhibitor suitable for use in the invention is a benzimidazole compound, for example, a benzimidazole compound described in the following patent documents U.S. Pat. Nos. 4,045,563; 4,255,431; 4,359,465; 4,472,409; 4,508,905; 4,628,098; 4,738,975; 5,045,321; 4,786,505; 4,853,230; 5,045,552, and 5,312,824; EP-A-295603; EP-A-166287; EP-A-519365; EP5129; EP 174,726; EP 166,287; GB 2,163,747; and JP-A-59181277.

In particular aspects of the invention a proton pump inhibitor comprises or is selected from the group consisting of omeprazole, hydroxyomeprazole, esomeprazole, tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, or prodrug thereof.

In particular aspects of the invention a proton pump inhibitor comprises or is selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, or prodrug thereof.

A "histamine-2 receptor antagonist" or "H-2 antagonist" refers to a compound which blocks H-2 receptors, but does not have meaningful activity in blocking histamine-1 receptors. Selective H-2 antagonists include compounds which are disclosed in US Pat. Nos. 5,294,433, 5,364,616, and US Patent Application No. 20050042283, including without limitation cimetidine [Merck Index, 11th edition (1989), p. 354 (entry no. 2279) and Physicians' Desk Reference, 46th edition (1992), p. 2228]; etintidine (U.S. Pat. No. 4,112,234); ranitidine or its hydrochloride salt (AH-19065) [U.S. Pat. No. 4,128,658, Merck Index, 11th edition (1989), p. 1291 (entry no. 8126), and Physicians' Desk Reference, 46th edition (1992), p. 1063]; hydroxymethyl ranitidine; ranitidine bismuth citrate (GR-122311, GR-122311X); AH-18801; N-cyano-N'-(2-(((5-((dimethylamino)methyl)-2-furanyl) methyl) thio)ethyl)-N"-methyl-guanidine; tiotidine (U.S. Pat. No. 4,165,378); ORF-17578 (U.S. Pat. No. 4,203,909); lupitidine (SKF-93479) (U.S. Pat. No. 4,234,588); donetidine (SKF-3574); famotidine (YM- 11170, MK-208) [Merck Index, 11th edition (1989), p. 617 (entry no. 3881), and Physicians' Desk Reference, 46th edition (1992), p. 1524]; roxatidine or rozatidine acetate [U.S. Pat. No. 4,293,557, Merck Index, 11 th edition (1989), p. 1316 (entry no. 8252)]; pifatidine; lamtidine (U.S. Pat. No. 4,318,913); BL-6548; BMY-25271; zaltidine (U.S. Pat. No. 4,374,843); nizatidine [U.S. Pat. No. 4,375,547, Merck Index, 11th edition (1989), p. 1052 (entry no. 6582), and Physicians' Desk Reference, 46th edition (1992), p. 1246)]; mifentidine and its hydrochloride salt (U.S. Pat. No. 4,386,099, Merck Index, 11th edition (1989), p. 973 (entry no. 6108)]; ICIA-5165 (U.S. Pat. No. 4,165,377); BMY-25368 (SKF-94482) (U.S. Pat. No. 4,390,701); SYF-94482; ICI-162846 (U.S. Pat. No. 4,451,463); ramixotidine (U.S. Pat. No. 4,474,790); BL-6341A (BMY-26539) (U.S. Pat. No.

4,394,508); Wy-45727 (U.S. Pat. No. 4,490,527); SR-58042 (U.S. Pat. No. 4,514,408); BMY-25405 (U.S. Pat. Nos. 4,528,377 and 4,600,779); loxtidine (U.S. Pat. No. 4,536,508); DA-4634 (U.S. Pat. Nos. 4,548,944 and 4,645,841); bisfentidine (U.S. Pat. No. 4,649,150); sufotidine (U.S. Pat. No. 4,670,448); ebrotidine (U.S. Pat. No. 4,728,755) HE-30-256 (U.S. Pat. No. 4,738,960); D-16637 (U.S. Pat. No. 4,738,983); FRG-8813 (U.S. Pat. Nos. 4,912,101 and 4,977,267); FRG-8701 (U.S. Pat. No. 4,837,316); impromidine (U.K. Patent Specification No. 1,531,237); L-643728 (European Patent Application No. 0,040,696); MK-208 (U.S. Pat. No. 4,283,408). and HB-408 (European Patent Application No. 0,186,275); burimamide, and metiamide.

The term, "growth/hormone regulatory factor" includes a large variety of growth factors and growth hormones, agents that modify one or more of the factors or hormones, and ligands and effectors for one or more receptors involved in binding of a growth factor or growth hormone as these terms are generally understood. The term includes analogs, derivatives, fragments and modifications of a wild-type growth/hormone regulatory factor and chimeric polypeptides comprising the factors. In aspects of the invention the term encompasses any polypeptide that shares substantial amino acid sequence identity with an endogenous mammalian growth factor or growth hormone and possesses a biological activity of a mammalian growth factor or growth hormone, as the case may be. In aspects of the invention a growth/hormone regulatory factor is related to pancreatic islet cell function, differentiation and/or proliferation. In particular a "growth/hormone regulatory factor" is selected that in combination with a gastrin agonist and optionally a gastrin compound results in one or more beneficial effect, more particularly a sustained beneficial effect. In an aspect of the invention a "growth/hormone regulatory factor" is selected that in combination with a gastrin agonist and optionally a gastrin compound results in one or more of increased C-peptide production, increased pancreatic insulin production, about normal or reduced blood glucose levels for a prolonged period following treatment, decreased insulin dependence or delivery, increased beta cell production and/or inhibition of programmed cell death (apoptosis), and/or reduction in insulin use in a subject.

Growth/hormone regulatory factors may be classified into various groups based on structural similarity of the peptides and proteins, functional similarity with respect to complementation of a gastrin agonist and optionally gastrin compound, functional similarity with respect to binding of one or more receptors, and these groups are each within the scope of various embodiments of the invention.

A growth/hormone regulatory factor may be selected for particular applications in the present invention based on one or more of the following characteristics: ability of the growth/hormone regulatory factor to bind to its receptor, preferably with an affinity constant K_d less than about 1 μM, more preferably less than about 100nM; ability to initiate a signal transduction pathway resulting in insulinotropic activity; insulinotropic activity; stimulation of beta cell proliferation/differentiation; resistance to DPP IV cleavage; and, an *in vivo* half-life of at least about 15 minutes to 24 hours, preferably 2 to 10 hours or 2 to 8 hours in humans using conventional methods (see for example, the method described in US 2003/0144206).

Examplary growth/hormone regulatory factors include but are not limited to: EGF receptor ligands including members of the EGF receptor ligand family such as betacellulin (BTC), transforming growth factor α (TGF-α), amphiregulin (AR), heparin binding-EGF (HB-EGF), epiregulin (EPR), and neuregulins 1-4 (NRG 1-4); PTH-related protein (PTHrP) receptor ligands such as PTHrP (PTHrP; Garcia- Ocana, A. et al., 2001, J. Clin. Endocrin. Metak. 86: 984-988); hepatocyte growth factor (HGF) receptor ligands such as HGF (HGF; Nielsen, J.

et al., 1999, J Mol Med 77: 62-66); fibroblast growth factors (FGF) such as FGF; keratinocyte growth factor (KGF) receptor ligands such as KGF; nerve growth factor (NGF) receptor ligands such as NGF; gastric inhibitory polypeptide (GIP) receptor ligands such as GIP; transforming growth factor beta (TGFβ) receptor ligands such as TGFβ (U.S. patent application 2002/0072115 published Jun. 13, 2002); laminin receptor ligands such as laminin-1; islet neogenesis associated protein (INGAP) receptor ligands such as INGAP; bone 15 morphogenetic factor (BMP) receptor ligands such as BMP-2; vasoactive intestinal peptide (VIP) receptor ligands such as VIP; glucagon-like peptide 1 receptor ligands such as GLP-1 and exendin-4; glucagon-like peptide 2 (GLP-2) receptor ligands such as GLP-2; dipeptidyl peptidase IV inhibitors; REG 20 receptor ligands such as REG protein; growth hormone (GH) receptor ligands such as GH; Prolactin (PRL) receptor ligands such as PRL and placental lactogen (PL); insulin-like growth factor (type 1 and 2) receptor ligands such as IGF1 and IGF-2; erythropoietin (EPO) receptor ligands such as EPO; Activin-A receptor ligands such as Activin-A; vascular endothelial growth factor (VEGF) receptor ligands such as VEGF; bone morphogenesis factor (BMP) receptor ligands such as BMP-2; vasoactive intestinal peptide (VIP) receptor ligands such as VIP; vascular endothelial growth factor (VEGF) receptor ligands such as VEGF; pituitary adenylate cyclase activating polypeptide (PACAP) receptor ligands such as PACAP; granulocyte colony stimulating factor (G-CSF) receptor ligands such as G-CSF; granulocyte-macrophage colony stimulating factor (GM-CSF) receptor ligands such as GM-CSH; platelet-derived growth factor (PDGF) receptor ligands such as PDGF; and, secretin receptor ligands such as secretin.

The term, "receptor ligand" as used herein in connection with a receptor for a particular ligand shall mean any composition that binds to, interacts with, or stimulates that receptor. A receptor ligand includes within the scope of the definition a receptor agonist for the receptor for any particular growth/hormone regulatory factor, whether or not the agonist is structurally related to the growth/hormone regulatory factor.

For any of the growth factors, enzymes, enzyme inhibitors, peptide, protein and hormone compounds herein that are indicated to be an exemplary growth/hormone regulatory factor, all known analogues and derivatives, whether naturally occurring or made by mutagenesis or designed and synthesized shall be considered equivalent to that growth/hormone regulatory factor.

Thus the term encompasses analogs of growth factors and growth hormones having deletions, insertions or substitutions and growth factors and growth hormones from other species and naturally occurring variants. The term "growth/hormone regulatory factor" also encompasses derivatives obtained by chemically modifying one or more amino acid residues of the parent peptide or analog thereof, for instance by alkylation, acylation, ester formation, amide formation, or by introducing a lipophilic functionality. Also considered among equivalents are conjugates, i.e., compositions derived by addition of one or more of a chemical group, and mixtures thereof. Further chimeric polypeptides comprising a growth factor or growth hormone are also encompassed within the term "growth/hormone regulatory factor".

Encoding genes may be altered by, for example, oligonucleotide directed mutagenesis to produce growth/hormone regulatory factor analogs thereof, such as the human recombinant analogs. Further, an identity or location of one, or more than one amino acid residue may be changed by targeted mutagenesis. The primary amino acid sequence of the protein may be augmented by conjugates, as by glycosylation, acylation, or by

addition of any other supplementary molecules, such as one or more of a lipid, a phosphate, a sulphate and/or an acetyl group. Further, individual amino acid residues in the chain may be modified by oxidation, reduction, or other derivatization. A growth/hormone regulatory factor may be cleaved to obtain any fragments which retain activity. A prodrug or a metabolite of a growth/hormone regulatory factor is equivalent to a growth/hormone regulatory factor. The whole polypeptide or protein or any fragment can be fused with any other peptide or protein such as immunoglobulins and other cytokines. Conjugates may include, for example, a composition comprising a growth/hormone regulatory factor coupled to a non-naturally occurring polymer comprising a polyethylene glycol moiety.

Alternatively, agents that induce synthesis of a growth/hormone regulatory factor or mimic the action of a growth/hormone regulatory factor are contemplated as equivalent compounds.

A growth/hormone regulatory factor can be prepared by synthetic, biological, or recombinant or chemical means, or obtained from commercial sources.

In aspects of the invention the growth/hormone regulatory factor is an epidermal growth factor (EGF) receptor ligand. The term "EGF receptor ligand" encompasses any compound, including peptides and non-peptide compounds, which fully or partially associate with and/or activate the EGF receptor. In aspects of the invention, an EGF receptor ligand is selected that has a suitable IC_{50}, for example an IC_{50} of about ~ 0.7 nM at an EGF receptor, as measured by methods known in the art (see Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin et al (1995) J. Biol. Chem. 270: 5019-5023 describing *in vitro* cell growth assays, and receptor binding assays as described in Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin et al (1995) J. Biol. Chem. 270: 5019-5023). An EGF receptor ligand may also be selected for particular applications in the present invention based on one or more of the following characteristics: ability of the EGF receptor ligand to bind to its receptor, preferably with an affinity constant K_d less than about 1 μM, more preferably less than about 100nM; ability to initiate a signal transduction pathway resulting in insulinotropic activity; insulinotropic activity; stimulation of beta cell proliferation/differentiation; resistance to DPP IV cleavage; and, an *in vivo* half-life in particular an *in vivo* half-life of at least about 15 minutes to 24 hours, preferably 2 to 10 hours or 2 to 8 hours in humans using conventional methods (see for example, the method described in US 2003/0144206).

In aspects of the invention the term encompasses compounds that in combination with a gastrin agonist and optionally gastrin compound provide at least one beneficial effect. In other aspects of the invention an EGF receptor ligand is selected to stimulate the EGF receptor such that when a gastrin agonist and/or gastrin compound in the same or adjacent tissue or in the same individual is also stimulated, neogenesis of insulin-producing pancreatic islet cells is induced. In a further aspect the term includes any EGF receptor ligands that demonstrate additive, synergistic, or complementary activity with a gastrin agonist and optionally a gastrin compound.

The term includes analogs, derivatives, fragments and modifications of a wild-type EGF receptor ligand, in particular EGF or TGFα. See also, Carpenter and Wahi, Chapter 4, in Peptide Growth Factors (Eds. Sporn and Roberts, Springer Verlag, 1990).

In aspects of the invention an EGF receptor ligand includes a polypeptide that shares a characteristic EGF-like domain defined by 6 cysteine residues that generate 3 peptide loops through the formation of disulphide bonds (Dunbar AJ and Goddard C, 2000 Int J. Biochem. Cell Biol. 32, 805-815). In aspect of the

invention an EGF receptor ligand includes a polypeptide that shares substantial amino acid sequence identity with a mammalian EGF and possesses some or all of the biological activity of a wild-type EGF. In aspects of the invention the EGF receptor ligand is a polypeptide that shares substantial amino acid sequence identity with full length, wild-type human epidermal growth factor (EGF; see SEQ ID NO: 36), a 53 amino acid protein with a molecular weight of 6217 daltons (Karnes, W., Epidermal growth factor and transforming growth factor alpha, 1994, Raven Press, New York).

The group of compounds that comprises the EGF receptor ligands further includes modified EGF receptor ligands which includes variants of normal or wild type EGF, TGF-α etc. Modifications may affect one or more biological activity such as the rate of clearance of EGF receptor ligand. The term includes peptides having an amino acid sequence substantially similar or identical to that of human EGF receptor ligand (e.g., EGF or TGF-α), for example, with one or a few amino acid substitutions at various residue positions. The term includes modified forms of an EGF receptor ligand which varying from wild-type EGF in chain length and amino acid sequence. Modifications can be made that affect both a biological activity and the rate of clearance of wild-type EGF receptor ligand.

In aspects of the invention an EGF receptor ligand includes a polypeptide that shares substantial amino acid sequence identity with a mammalian TGFα and possesses some or all of the biological activity of a wild-type TGFα. Amino acid sequences of human TGF-α are shown in SEQ ID NOs: 42 and 43 and in GenBank Accession No. NP_003227. TGF-α shares cysteine disulfide bond structures with a family of TGF-α related polypeptides such as vaccinia growth factor, amphiregulin precursor, betacellulin precursor, betacellulin, heparin binding EGF-like growth factor, epiregulin (rodents), HUS 19878, myxomavirus growth factor (MGF), Shope fibroma virus growth factor (SFGF), and schwannoma derived growth factor. Such TGF-α related polypeptides, including without limitation amphiregulin, vaccinia growth factor, myxomavirus growth factor (MGF), pox virus growth factor, Shope fibroma virus growth factor (SFGF), heparin-binding EGF-like growth factor (HB-EGF) are also useful in the methods of the invention.

The invention also provides a class of peptides, including EGF and peptides smaller than the 53 amino acid human EGF, yet retaining some or all EGF biological activity, which are useful as pharmacologic and therapeutic agents. Thus, examples of EGF receptor ligands include without limitation full length EGF, which is EGF1-53, and further include EGF1-48, EGF1-49, EGF1-52, and fragments and active analogs thereof (see for example US Pat. No. 5,434,135).

The invention also provides a class of peptides, including TGF-α and peptides smaller than the 50 amino acid human TGF-α [e.g., SEQ ID NOs. 42 and 43, GenBank Accession No. NP_003227], yet retaining some or all TGF-α biological activity, which are useful as pharmacologic and therapeutic agents. Examples of these peptides include without limitation TGE-α forms TGF-α1-48, TGF-α1-47, TGFα1-51, and TGF-α57 (US 6815418).

Recombinant EGF forms have been genetically engineered to have alterations in structure and activities, for example, EGF having a methionine at position 21 replaced by a leucine residue has been described (U.S. patent number 4,760,023). Recombinant human EGF (hEGF) having 51 residues, i.e., lacking the two C-terminal residues at positions 52 and 53 of hEGF, and having a neutral amino acid substitution at position 51, retain EGF activity and are more resistant to protease degradation during a microbial production process, and following

administration to a subject. A series of nucleic acid molecules have been described that encode a family of proteins that have significant similarity to EGF and TGFα (WO 00/29438). EGF muteins (mutated EGF) having histidine at residue 16 replaced with a neutral or acidic amino acid have been described (WO 93/03757), such forms retaining activity at low values of pH.

Chemical analogs and fragments of EGF and TGFα retain ability to bind various members of the EGF receptor family (U.S. patent number 4,686,283). Various modifications of EGF or TGFα confer advantageous properties affecting one or more of recombinant protein production, *in vitro* and *in vivo* stability, and *in vivo* activity.

In aspects of the invention, a recombinant modified EGF receptor ligand is employed comprising a C-terminus deleted form of human EGF of 51 amino acids in length, having asparagine at position 51 (referred to herein as EGF51N), which retains substantial activity in the treatments described herein, and has *in vivo* and/or *in vitro* stability that is at least about as great or greater than normal or wild type hEGF (See International Application No. PCT/US02/33907).

In aspects of the invention, an EGF receptor ligand is a recombinant EGF having a methionine at position 21 replaced by a leucine residue (U.S. Pat. No. 4,760,023); a recombinant hEGF with an aspartyl residue at position 11 replaced by an isoaspartyl (George-Nascimento et al., Biochemistry, 29, 9584-9591, 1990); an EGF mutein having histidine at residue 16 replaced with a neutral or acidic amino acid (WO 93/03757), or a chemical analog or fragment of wild-type EGF and TGF-alpha that binds to members of the EGF receptor family (See U.S. Pat. No. 4,686,283). In another aspect, an EGF receptor ligand is a polypeptide that has substantial sequence identity or similarity to EGF and TGF-alpha and is described in WO 00/29438.

In particular aspects of the invention an EGF receptor ligand is a wild-type EGF receptor ligand with modifications to the C-terminus amino acid residues at position 48 to position 53. Examples of such modified EGF receptor ligands are described in US Published Application No. 20030171269 and WO 03040310. In other aspects, an EGF receptor ligand is a modified EGF receptor ligand including without limitation a wild-type EGF receptor ligand with amino acid residues at position 48 to position 53 deleted or replaced. In a particular aspect, an EGF receptor ligand is a wild-type EGF receptor ligand with the following amino acids deleted or replaced: the basic amino acids at positions 48 (lysine in wild-typeEGF) and 53 (arginine), the aromatic amino acids at positions 49 (tryptophan) and 50 (tryptophan), and/or the aliphatic amino acid at position 52 (leucine).

In an aspect of the invention, an EGF receptor ligand is represented by A-B wherein A comprises an amino acid sequence substantially identical to amino acids 1 to 47, 1 to 48, 1 to 50, or 1 to 53 of SEQ ID NO:36 through 40, and B is between 1 to 10 amino acids. In a particular aspect, B is a single neutral, hydrophobic or charged amino acid. In another particular aspect, B is a single amino acid excluding glutamate. In further particular aspects, B is a single neutral, hydrophobic or charged amino acid.

In an embodiment B is a hydrophobic amino acid such as alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, praline, tryptophan, tyrosine, or valine.

In another embodiment B is a charged amino acid such as aspartic acid, glutamic acid, arginine, or lysine.

In a further embodiment B is a neutral amino acid such as asparagine, glutamine, cysteine, glycine, isoleucine, leucine, methionine, phenylalanine, praline, alanine, threonine, tryptophan, tyrosine, or serine, especially asparagine, glutamine, alanine, or serine.

In aspects of the invention a EGF receptor ligand represented by A-B is selected to provide increased resistance to proteolysis compared with that of wild-type EGF and/or a biological activity that is least 75%, 80%, 85%,90%, 95%, 98%, or 99% compared with that of wild-type EGF. The biological activity can be mitogenesis, cytoprotection, inhibition of acid secretion, growth of a tissue precursor cell, differentiation of a tissue precursor cell, and/or growth and differentiation of a tissue precursor cell. Mitogenesis can be determined by the effect of the amino acid sequence on rate of mitosis of epithelial cells. Acid secretion can be determined in a gastric fistulated animal. Differentiation can be determined by islet neogenesis or mucosal cell formation

In a particular embodiment, an EGF receptor ligand is utilized wherein A comprises amino acids 1-50 of SEQ ID NO: 36 wherein at least one of residues 1-50 is substituted with at least one conservative amino acid substitution, especially one, two or three amino acid substitutions, in the sequence as shown in SEQ ID NO: 36. A conservative amino acid substitution refers to the replacement of an amino acid with a chemically similar amino acid. Examples of conservative amino acid substitution are: a charged amino acid replaced by a different amino acid of the same charge, such as Asp replaced by Glu, or an aromatic hydrophobic amino acid, e.g., Trp, replaced by a different aromatic hydrophobic amino acid, e.g., Phe (see U.S. Pat. No. 6,207,154, issued Mar. 27, 2001).

An EGF receptor ligand can be utilized wherein A comprises amino acids 1 to 47, 1 to 48, 1 to 50, or 1 to 53 of SEQ ID NO: 36 through 40 and wherein amino acids 1 to 47, 1 to 48, 1 to 50, or 1 to 53 comprise a deletion of at least one amino acid residue from positions 1-5 as shown in SEQ ID NO: 36 through 40. Such an EGF receptor ligand may have at least 50% of a biological activity of human wild-type EGF as shown in SEQ ID NO: 36.

In another particular embodiment, an EGF receptor ligand is utilized wherein A comprises amino acids 1-53 of SEQ ID NO: 36 and wherein at least one amino acid is replaced at positions 48-53 of the carboxy terminus, the amino acid sequence being more stable to proteolysis than that of SEQ ID NO: 36.

In a further particular embodiment, an EGF receptor ligand is utilized wherein A comprises amino acids 1-50 of SEQ ID NO: 36 and B is aspargine (i.e., EGF 1-51 $glu^{51}asn$ Asn^{51}-hEGF51, or EGF51N) (see SEQ ID NO. 37 and the DNA sequence of SEQ ID NO. 41 encoding an EGF51N).

In a further particular embodiment, an EGF receptor ligand is utilized wherein A comprises amino acids 1-50 of SEQ ID NO: 36 and B is alanine (EGF51A - SEQ ID NO. 38).

In a further particular embodiment, an EGF receptor ligand is utilized wherein A comprises amino acids 1-50 of SEQ ID NO: 36 and B is glutamine (EGFQ - SEQ ID NO. 39).

In a further particular embodiment, an EGF receptor ligand is utilized wherein A comprises amino acids 1-50 of SEQ ID NO: 36 and B is serine (EGF51S - SEQ ID NO. 40).

An EGF receptor ligand can be prepared using methods known in the art, in particular using recombinant techniques. In an aspect, an EGF receptor ligand is produced using recombinant techniques in an appropriate host cell for example the organism *Pichia pastoris*. In an embodiment, an EGF receptor ligand has a

purity of greater than 80%, 83%, 85%, 86%, 90%, 92%, 94%, 95%, 98%, or 99%; a concentration of about 1.0 to 1.5 mg/ml, and/or a molecular weight of about 5932 ± 3 Da.

In aspects of the invention the growth/hormone regulatory factor is a GLP-1 agonist. A "GLP-1 agonist" is understood to refer to any compound, including peptides and non-peptide compounds, which fully or partially activate a GLP-1 receptor (e.g, human GLP-1 receptor), or fully or partially mimic or increase a reaction, activity, or function of glucagon-like peptide 1 receptor ligands or initiate such reaction, activity, or function, or reduce or prevent inhibition of any reaction, activity or function of glucagon-like peptide 1 receptor ligands. In particular aspects, the "GLP-1 agonist" is any peptide or non-peptide small molecule that binds to a GLP-1 receptor, preferably with an affinity constant (K_D) or a potency (EC_{50}) of below 1 μM, e.g., below 100 nM as measured by methods known in the art (see e.g. WO 98/08871) and exhibits insulinotropic activity, where insulinotropic activity may be measured *in vivo* or *in vitro* assays known to those of ordinary skill in the art. For example, the GLP-1 agonist may be administered to an animal and the insulin concentration measured over time.

Representative GLP-1 agonists are listed in Table 1. GLP-1 agonists are in each case generically and specifically disclosed in the referenced patent document or publication. Any of the substances disclosed in the patent documents and publications referenced in Table 1 are considered potentially useful as GLP-1 agonists to be used in carrying out the present invention.

In an embodiment, the GLP-1 agonist comprises or is selected from the group of glucagon-like peptide 1 receptor ligands consisting of GLP-1(7-36)-amide, GLP-1(7-37), a GLP-1(7-36)-amide analogue, a GLP-1(7-37) analogue, or a derivative of any of these.

Methods for identifying GLP-1 agonists are described in WO 93/19175 (Novo Nordisk A/S) and examples of suitable GLP-1 analogues and derivatives which can be used according to the present invention include those referred to in WO 99/43705 (Novo Nordisk A/S), WO 99/43706 (Novo Nordisk A/S), WO 99/43707 (Novo Nordisk A/S), WO 98/08871 (Novo Nordisk A/S), WO 99/43708 (Novo Nordisk A/S), WO 99/43341 (Novo Nordisk A/S), WO 87/06941 (The General Hospital Corporation), WO 90/11296 (The General Hospital Corporation), WO 91/11457 (Buckley et al.), WO 98/43658 (Eli Lilly & Co.), EP 0708179-A2 (Eli Lilly & Co.), and EP 0699686-A2 (Eli Lilly & Co.), WO 01/98331 (Eli Lilly & Co).

In an embodiment, the GLP-1 agonist comprises a parent polypeptide of the formula GLP-1(7-R) wherein R is 36, 37, 38, 39, 40, 41, 42, 43, 44, and 45, and wherein optionally up to 5, 10, or 15 amino acid residues are replaced with any α-amino acid residue.

In an aspect, a GLP-1 agonist comprises or is selected from the group of glucagon-like peptide 1 receptor ligands consisting of GLP-1(7-36)-amide, GLP-1(7-37), a GLP-1(7-36)-amide analogue, a GLP-1(7-37) analogue, or a derivative of any of these. In particular embodiments of the invention a GLP-1 agonist is a GLP-1(7-36)-amide or Tyr^{31}-exendin-4(1-31)-amide.

In another aspect, the GLP-1 agonist is a naturally truncated GLP-1 polypeptide (GLP-1(7-36) or ((GLP-1(7-37)), or an analogue or derivative thereof. The sequences of these naturally occurring truncated GLP-1 agonists are represented in SEQ ID NOs. 18, 19, and 20.

In certain aspects of the invention, a GLP-1 agonist may have the amino acid sequence of SEQ ID NOs. 17, 18, or 19 modified so that amino acid residues at positions 1-20, preferably 1-15, more preferably 1-10, most preferably 1-5 differ from the sequences of SEQ ID NOs. 17, 18 or 19.

In an embodiment of the invention, the GLP-1 agonist is an analogue of GLP-1(7-37) or GLP-1(7-36) which has less than 10 amino acid residues that are different from those in GLP-1(7-37) or GLP-1(7-36), less than 5 amino acid residues that are different from those in GLP-1(7-37) or GLP-1(7-36), less than 3 amino acid residues that are different from those in GLP-1 (7-37) or GLP-1(7-36), preferably only one amino acid residue that is different from a sequence of GLP-1(7-37) or GLP-1(7-36).

GLP-1 agonists that may have specific utility in the present invention include polypeptides where one or more amino acids have been added to the N-terminus and/or C-terminus of GLP-1(7-37) or GLP-1(7-36). Preferably, about one to six amino acids may be added to the N-terminus and/or from about one to eight amino acids may be added to the C-terminus. In certain applications GLP-1 agonists are selected that have up to 39 amino acids. Amino acids at positions 1-6 of an extended GLP-1 agonist may be selected so that they are the same or are conservative substitutions of the amino acids at the corresponding positions of the parent GLP-1(7-37) or GLP-1(7-36). Amino acids at positions 38-45 of an extended GLP-1 agonist may be selected so that they are the same or conservative substitutions of the amino acids at the corresponding positions of exendin-3 or exendin-4 (SEQ ID NO. 21 and 22, respectively).

In aspects of the invention a GLP-1 agonist is utilized comprising a position 8 analogue wherein the backbone for such analogs or fragments thereof contains an amino acid other than alanine. The amino acid at position 8 may be selected from glycine, valine, leucine, isoleucine, serine, threonine, or methionine.

In an embodiment a GLP-1 agonist is an insulinotropic analogue of GLP-1(1-37), for example, Met^{8}-GLP-1(7-37), wherein the alanine in position 8 has been replaced by methionine and the amino acid residues in position 1 to 6 have been deleted, and Arg^{34}-GLP-1(7-37) wherein the valine in position 34 has been replaced with arginine and the amino acid residues in position 1 to 6 have been deleted.

In another embodiment, GLP-1 agonists are selected comprising the sequence GLP-1(7-37)OH and GLP-1(7-36) amide, and the corresponding position 8 analogs wherein the backbone for such analogs contains an amino acid other than alanine. The amino acid at position 8 may be selected from glycine, valine, leucine, isoleucine, serine, threonine, or methionine, preferably valine or glycine. The analogs may additionally contain (a) an amino acid at position 22 selected from glutamic acid, lysine, aspartic acid, arginine, and preferably glutamic acid or lysine; (b) an amino acid at position 30 selected from glutamic acid, aspartic acid, serine, or histidine; (c) an amino acid at position 37 selected from lysine, arginine, threonine, glutamic acid, aspartic acid, serine, tryptophan, tyrosine, phenylalanine, or histidine; and/or (d) amino acid at position 27 selected from alanine, lysine, arginine, tryptophan, tyrosine, phenylalanine, or histidine.

A group of GLP-1 analogs and derivatives for use in the present invention comprises the GLP-1 agonists described in U.S. Pat. No. 5,545,618 and US Patent Application Serial No. 20040018975. The analogs include active GLP-1 peptides, 7-34, 7-35, 7-36 and 7-37 having amino acid substitutions at positions 7-10 and/or are truncations at the C-terminus and/or contain various other amino acid substitutions in the basic peptide. Preferred analogs include those with D-amino acid substitutions in the 7 and 8 positions and/or N-alkylated or N-acylated amino acids in the 7 position since they are particularly resistant to degradation *in vivo*.

In aspects of the invention, a GLP-1 agonist comprises a peptide comprising or selected from the group consisting of GLP-1 (1-38); GLP-1 (1-39), GLP-1 (1-40), GLP-1 (1-41), GLP-1 (7-38), GLP-1 (7-39), GLP-1 (7-40), and GLP-1 (7-41).

In another aspect of the invention at least one amino acid of a GLP-1 agonist has at least one substituent attached directly or indirectly (e.g. via a spacer such as γ-Glu or β-Ala). A substituent is generally selected to make the profile of action of the parent GLP-1 agonist more protracted, make the GLP-1 agonists more metabolically and physically stable, and/or increase solubility of the GLP-1 agonist. An example of a particular substituent is an amide, a carbohydrate, and a lipophilic substituent. A lipophilic substituent includes but is not limited to an alkyl group, a group which has an ω-carboxylic acid group, an acyl group of a straight-chain or branched fatty acid or alkane such as tetradecanoyl, hexadecanoyl. Particular compositions, conjugates and treatments of the invention use GLP-1 agonists with lipophilic substitutents such as those described in WO 99/43341 (Novo Nordisk) and US 2003/0119734A1 (Novo Nordisk).

In particular aspects of the invention a GLP-1 agonist is a GLP-1(7-36)-amide or Tyr^{31}-exendin-4(1-31)-amide.

In one embodiment, the GLP-1 agonist is a derivative of GLP-1(7-36)-amide, GLP-1(7-37), a GLP-1(7-36)-amide analogue or a GLP-1(7-37) analogue, which comprises at least one lipophilic substituent.

In embodiments of the invention, the GLP-1 derivative has three lipophilic substituents, two lipophilic substituents, or one lipophilic substituent attached to the parent peptide (ie GLP-1(7-36)-amide, GLP-1(7-37), a GLP-1(7-36)-amide analogue or a GLP-1(7-37) analogue), where each lipophilic substituent(s) preferably has 4-40 carbon atoms, more preferably 8-30 carbon atoms, even more preferably 8-25 carbon atoms, even more preferably 12-25 carbon atoms, and most preferably 14-18 carbon atoms.

Certain aspects of the invention provide a GLP-1 agonist that is a derivative of GLP-1 (7-36) or GLP-1 (7-37) comprising a lipophilic substitutent. In an embodiment, the GLP-1 agonist is $Arg^{34}Lys^{26}(N^{\varepsilon}(\gamma\text{-Glu}(N^{\alpha}\text{-hexadecanoyl})))$-GLP-1(7-37).

In one embodiment, the lipophilic substituent comprises a partially or completely hydrogenated cyclopentanophenathrene skeleton.

In another embodiment, the lipophilic substituent is a straight-chain or branched alkyl group.

In yet another embodiment, the lipophilic substituent is an acyl group of a straight-chain or branched fatty acid. Preferably, the lipophilic substituent is an acyl group having the formula $CH_3(CH_2)_nCO$-, wherein n is an integer from 4 to 38, preferably an integer from 12 to 38, and most preferably is $CH_3(CH_2)_{12}CO$-, $CH_3(CH_2)_{14}CO$-, $CH_3(CH_2)_{16}CO$-, $CH_3(CH_2)_{18}CO$-, $CH_3(CH_2)_{20}CO$- and $CH_3(CH_2)_{22}CO$-. In a more preferred embodiment, the lipophilic substituent is tetradecanoyl. In a most preferred embodiment, the lipophilic substituent is hexadecanoyl.

In a further embodiment of the present invention, the lipophilic substituent has a group which is negatively charged such as a carboxylic acid group. For example, the lipophilic substituent may be an acyl group of a straight-chain or branched alkane α,ω-dicarboxylic acid of the formula $HOOC(CH_2)_mCO$-, wherein m is an integer from 4 to 38, preferably an integer from 12 to 38, and most preferably is $HOOC(CH_2)_{14}CO$-, $HOOC(CH_2)_{16}CO$-, $HOOC(CH_2)_{18}CO$-, $HOOC(CH_2)_{20}CO$- or $HOOC(CH_2)_{22}CO$-.

In selected GLP-1 derivatives of the invention, the lipophilic substituent(s) contain a functional group which can be attached to one of the following functional groups of an amino acid of the parent GLP-1 peptide:

(a) the amino group attached to the alpha-carbon of the N-terminal amino acid,

(b) the carboxy group attached to the alpha-carbon of the C-terminal amino acid,

(c) the epsilon-amino group of any Lys residue,

(d) the carboxy group of the R group of any Asp and Glu residue,

(e) the hydroxy group of the R group of any Tyr, Ser and Thr residue,

(f) the amino group of the R group of any Trp, Asn, Gln, Arg, and His residue, or

(g) the thiol group of the R group of any Cys residue.

In one embodiment, a lipophilic substituent is attached to the carboxy group of the R group of any Asp and Glu residue.

In another embodiment, a lipophilic substituent is attached to the carboxy group attached to the alpha-carbon of the C-terminal amino acid.

In a preferred embodiment, a lipophilic substituent is attached to the epsilon-amino group of any Lys residue.

In a preferred embodiment of the invention, the lipophilic substituent is attached to the parent GLP-1 peptide by means of a spacer. A spacer must contain at least two functional groups, one to attach to a functional group of the lipophilic substituent and the other to a functional group of the parent GLP-1 peptide.

In one embodiment, the spacer is an amino acid residue except Cys or Met, or a dipeptide such as Gly-Lys. For purposes of the present invention, the phrase "a dipeptide such as Gly-Lys" means any combination of two amino acids except Cys or Met, preferably a dipeptide wherein the C-terminal amino acid residue is Lys, His or Trp, preferably Lys, and the N-terminal amino acid residue is Ala, Arg, Asp, Asn, Gly, Glu, Gln, Ile, Leu, Val, Phe, Pro, Ser, Tyr, Thr, Lys, His and Trp. Preferably, an amino group of the parent peptide forms an amide bond with a carboxylic group of the amino acid residue or dipeptide spacer, and an amino group of the amino acid residue or dipeptide spacer forms an amide bond with a carboxyl group of the lipophilic substituent.

Preferred spacers are lysyl, glutamyl, asparagyl, glycyl, beta-alanyl and gamma-aminobutanoyl, each of which constitutes an individual embodiment. Most preferred spacers are glutamyl and beta-alanyl. When the spacer is Lys, Glu or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the amino group thereof may form an amide bond with a carboxyl group of the lipophilic substituent. When Lys is used as the spacer, a further spacer may in some instances be inserted between the ε-amino group of Lys and the lipophilic substituent. In one embodiment, such a further spacer is succinic acid which forms an amide bond with the ε-amino group of Lys and with an amino group present in the lipophilic substituent. In another embodiment such a further spacer is Glu or Asp which forms an amide bond with the ε-amino group of Lys and another amide bond with a carboxyl group present in the lipophilic substituent, that is, the lipophilic substituent is an N^{ε}-acylated lysine residue.

In another embodiment, the spacer is an unbranched alkane α,ω-dicarboxylic acid group having from 1 to 7 methylene groups, which spacer forms a bridge between an amino group of the parent peptide and an amino group of the lipophilic substituent. Preferably, the spacer is succinic acid.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula $CH_3(CH_2)_pNH\text{-}CO(CH_2)_qCO\text{-}$, wherein p is an integer from 8 to 33, preferably from 12 to 28 and q is an integer from 1 to 6, preferably 2.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula $CH_3(CH_2)_rCO$-NHCH(COOH)$(CH_2)_2CO$-, wherein r is an integer from 4 to 24, preferably from 10 to 24.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula $CH_3(CH_2)_sCO$-NHCH($(CH_2)_2$COOH)CO-, wherein s is an integer from 4 to 24, preferably from 10 to 24.

In a further embodiment, the lipophilic substituent is a group of the formula $COOH(CH_2)_tCO$- wherein t is an integer from 6 to 24.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-$CO(CH_2)_uCH_3$, wherein u is an integer from 8 to 18.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula $CH_3(CH_2)_vCO$-NH-$(CH_2)_z$-CO, wherein v is an integer from 4 to 24 and z is an integer from 1 to 6.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-COCH($(CH_2)_2$COOH)NH-$CO(CH_2)_wCH_3$, wherein w is an integer from 10 to 16.

In a further embodiment, the lipophilic substituent with the attached spacer is a group of the formula -NHCH(COOH)$(CH_2)_4$NH-$CO(CH_2)_2$CH(COOH)NHCO$(CH_2)_xCH_3$, wherein x is zero or an integer from 1 to 22, preferably 10 to 16.

In yet another embodiment the GLP-1 agonist is Arg^{34}, $Lys^{26}(N^{\varepsilon}$-(γ-Glu(N^{α}-hexadecanoyl)))-GLP-1(7-37).

In yet another embodiment the GLP-1 agonist comprises or is selected from the group consisting of Gly^8-GLP-1(7-36)-amide, Gly^8-GLP-1(7-37), Val^8-GLP-1(7-36)-amide, Val^8-GLP-1(7-37), Val^8Asp^{22}-GLP-1(7-36)-amide, Val^8Asp^{22}-GLP-1(7-37) , Val^8Glu^{22}-GLP-1(7-36)-amide , Val^8Glu^{22}-GLP-1(7-37), Val^8Lys^{22}-GLP-1(7-36)-amide, Val^8Lys^{22}-GLP-1(7-37), Val^8Arg^{22}-GLP-1(7-36)-amide, Val^8Arg^{22}-GLP-1(7-37), Val^8His^{22}-GLP-1(7-36)-amide, Val^8His^{22}-GLP-1(7-37), analogues thereof and derivatives of any of these.

In yet another embodiment the GLP-1 agonist comprises or is selected from the group consisting of Arg^{26}-GLP-1(7-37); Arg^{34}-GLP-1(7-37); Lys^{36}-GLP-1(7-37); $Arg^{26,34}Lys^{36}$-GLP-1(7-37); $Arg^{26,34}$-GLP-1(7-37); $Arg^{26,34}Lys^{40}$-GLP-1(7-37); $Arg^{26}Lys^{36}$-GLP-1(7-37); $Arg^{34}Lys^{36}$-GLP-1(7-37); Val^8Arg^{22}-GLP-1(7-37); Met^8Arg^{22}-GLP-1(7-37);Gly^8His^{22}-GLP-1(7-37); Val^8His^{22}-GLP-1(7-37); Met^8His^{22}-GLP-1(7-37);His^{37}-GLP-1(7-37); Gly^8-GLP-1(7-37); Val^8-GLP-1(7-37); Met^8-GLP-1(7-37);Gly^8Asp^{22}-GLP-1(7-37); Val^8Asp^{22}-GLP-1(7-37); Met^8Asp^{22}-GLP-1(7-37);Gly^8Glu^{22}-GLP-1(7-37); Val^8Glu^{22}-GLP-1(7-37); Met^8Glu^{22}-GLP-1(7-37); Gly^8Lys^{22}-GLP-1(7-37); Val^8Lys^{22}-GLP-1(7-37); Met^8Lys^{22}-GLP-1(7-37); Gly^8Arg^{22}-GLP-1(7-37); $Val^8Lys^{22}His^{37}$-GLP-1(7-37); $Gly^8Glu^{22}His^{37}$-GLP-1(7-37); $Val^8Glu^{22}His^{37}$-GLP-1(7-37); $Met^8Glu^{22}His^{37}$-GLP-1(7-37);Gly^8Lys^{22} His^{37}-GLP-1(7-37); $Met^8Lys^{22}His^{37}$-GLP-1(7-37);$Gly^8Arg^{22}His^{37}$-GLP-1(7-37); $Val^8Arg^{22}His^{37}$-GLP-1(7-37); $Met^8Arg^{22}His^{37}$-GLP-1(7-37); $Gly^8His^{22}His^{37}$-GLP-1(7-37); $Val^8His^{22}His^{37}$-GLP-1(7-37); $Met^8His^{22}His^{37}$-GLP-1(7-37); Gly^8His^{37}-GLP-1(7-37); Val^8His^{37}-GLP-1(7-37); Met^8His^{37}-GLP-1(7-37);Gly^8Asp^{22} His^{37}-GLP-1(7-37); $Val^8Asp^{22}His^{37}$-GLP-1(7-37); $Met^8Asp^{22}His^{37}$-GLP-1(7-37); Arg^{26}-GLP-1(7-36)-amide; Arg^{34}-GLP-1(7-36)-amide; Lys^{36}-GLP-1(7-36)-amide; $Arg^{26,34}Lys^{36}$-GLP-1(7-36)-amide; $Arg^{26,34}$-GLP-1(7-36)-amide; $Arg^{26,34}Lys^{40}$-GLP-1(7-36)-amide; $Arg^{26}Lys^{36}$-GLP-1(7-36)-amide; $Arg^{34}Lys^{36}$-GLP-1(7-36)-amide; Gly^8-GLP-1(7-36)-amide; Val^8-GLP-1(7-36)-amide; Met^8-GLP-1(7-36)-amide;Gly^8Asp^{22}-GLP-1(7-36)-amide; $Gly^8Glu^{22}His^{37}$-GLP-1(7-36)-amide; Val^8Asp^{22}-GLP-1(7-36)-amide; Met^8Asp^{22}-GLP-1(7-36)-amide;Gly^8Glu^{22}-GLP-1(7-36)-amide; Val^8Glu^{22}-GLP-1(7-36)-amide; Met^8Glu^{22}-GLP-1(7-36)-amide;

Gly^8Lys^{22}-GLP-1(7-36)-amide; Val^8Lys^{22}-GLP-1(7-36)-amide; Met^8Lys^{22}-GLP-1(7-36)-amide; $Gly^8His^{22}His^{37}$-GLP-1(7-36)-amide; Gly^8Arg^{22}-GLP-1(7-36)-amide; Val^8Arg^{22}-GLP-1(7-36)-amide; Met^8Arg^{22}-GLP-1(7-36)-amide;Gly^8His^{22}-GLP-1(7-36)-amide; Val^8His^{22}-GLP-1(7-36)-amide; Met^8His^{22}-GLP-1(7-36)-amide;His^{37}-GLP-1(7-36)-amide; $Val^8Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Arg^{22}His^{37}$-GLP-1(7-36)-amide; Gly^8His^{37}-GLP-1(7-36)-amide; Val^8His^{37}-GLP-1(7-36)-amide; Met^8His^{37}-GLP-1(7-36)-amide;Gly^8Asp^{22} His^{37}-GLP-1(7-36)-amide; $Val^8Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8Glu^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Glu^{22}His^{37}$-GLP-1(7-36)-amide;Gly^8Lys^{22} His^{37}-GLP-1(7-36)-amide; $Val^8Lys^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Lys^{22}His^{37}$-GLP-1(7-36)-amide;$Gly^8Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8His^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8His^{22}His^{37}$-GLP-1(7-36)-amide; and derivatives thereof.

In embodiments of the invention, the GLP-1 agonist comprises or is selected from the group consisting of: Gly^8-GLP-1(7-36)-amide, Gly^8-GLP-1(7-37), Val^8-GLP-1(7-36)-amide, Val^8-GLP-1(7-37), Val^8Asp^{22}-GLP-1(7-36)-amide, Val^8Asp^{22}-GLP-1(7-37), Val^8Glu^{22}-GLP-1(7-36)-amide, Val^8Glu^{22}-GLP-1(7-37), Val^8Lys^{22}-GLP-1(7-36)-amide, Val^8Lys^{22}-GLP-1(7-37) -Val^8Arg^{22}-GLP-1(7-36)-amide, Val^8Arg^{22}-GLP-1(7-37), Val^8His^{22}-GLP-1(7-36)-amide, -Val^8His^{22}-GLP-1(7-37), Arg^{26}-GLP-1(7-37); Arg^{34}-GLP-1(7-37); Lys^{36}-GLP-1(7-37); $Arg^{26,34}Lys^{36}$-GLP-1(7-37); $Arg^{26,34}$-GLP-1(7-37); $Arg^{26,34}Lys^{40}$-GLP-1(7-37); $Arg^{26}Lys^{36}$-GLP-1(7-37); $Arg^{34}Lys^{36}$-GLP-1(7-37); Val^8Arg^{22}-GLP-1(7-37); Met^8Arg^{22}-GLP-1(7-37);Gly^8His^{22}-GLP-1(7-37); Val^8His^{22}-GLP-1(7-37); Met^8His^{22}-GLP-1(7-37);His^{37}-GLP-1(7-37); Gly^8-GLP-1(7-37); Val^8-GLP-1(7-37); Met^8-GLP-1(7-37);Gly^8Asp^{22}-GLP-1(7-37); Val^8Asp^{22}-GLP-1(7-37); Met^8Asp^{22}-GLP-1(7-37); Gly^8Glu^{22}-GLP-1(7-37); Val^8-GLP-1(7-37); Gly^8Lys^{22}-GLP-1(7-37); Val^8Lys^{22}-GLP-1(7-37); $Glu^{22}Met^8Glu^{22}$-GLP-1(7-37); Gly^8Lys^{22}-GLP-1(7-37); Val^8Lys^{22}-GLP-1(7-37); Met^6Lys^{22}-GLP-1(7-37); Gly^8Arg^{22}-GLP-1(7-37); $Val^8Lys^{22}His^{37}$-GLP-1(7-37); $Gly^8Glu^{22}His^{37}$-GLP-1(7-37); $Val^8Glu^{22}His^{37}$-GLP-1(7-37); $Met^8Glu^{22}His^{37}$-GLP-1(7-37);Gly^8Lys^{22} His^{37}-GLP-1(7-37); $Met^8Lys^{22}His^{37}$-GLP-1(7-37);$Gly^8Arg^{22}His^{37}$-GLP-1(7-37); $Val^8Arg^{22}His^{37}$-GLP-1(7-37); $Met^8Arg^{22}His^{37}$-GLP-1(7-37); $Gly^8His^{22}His^{37}$-GLP-1(7-37); $Val^8His^{22}His^{37}$-GLP-1(7-37); $Met^8His^{22}His^{37}$-GLP-1(7-37); Gly^8His^{37}-GLP-1(7-37); Val^8His^{37}-GLP-1(7-37); Met^8His^{37}-GLP-1(7-37); $Gly^8Asp^{22}His^{37}$-GLP-1(7-37); $Val^8Asp^{22}His^{37}$-GLP-1(7-37); $Met^8Asp^{22}His^{37}$-GLP-1(7-37); Arg^{26}-GLP-1(7-36)-amide; Arg^{34}-GLP-1(7-36)-amide; Lys^{36}-GLP-1(7-36)-amide; $Arg^{26,34}Lys^{36}$-GLP-1-(7-36)-amide; $Arg^{26,34}$-GLP-1(7-36)-amide; $Arg^{26,34}Lys^{40}$-GLP-1(7-36)-amide; $Arg^{26}Lys^{36}$-GLP-1(7-36)-amide; $Arg^{34}Lys^{36}$-GLP-1(7-36)-amide; Gly^8-GLP-1(7-36)-amide; Val^8-GLP-1(7-36)-amide; Met^8-GLP-1(7-36)-amide;Gly^8Asp^{22}-GLP-1(7-36)-amide; $Gly^8Glu^{22}His^{37}$-GLP-1(7-36)-amide; Val^8Asp^{22}-GLP-1(7-36)-amide; Met^8Asp^{22}-GLP-1(7-36)-amide;Gly^8Glu^{22}-GLP-1(7-36)-amide; Val^8Glu^{22}-GLP-1(7-36)-amide; Met^8Glu^{22}-GLP-1(7-36)-amide; Gly^8Lys^{22}-GLP-1(7-36)-amide; Val^8Lys^{22}-GLP-1(7-36)-amide; Met^8Lys^{22}-GLP-1(7-36)-amide; $Gly^8His^{22}His^{37}$-GLP-1(7-36)-amide; Gly^8Arg^{22}-GLP-1(7-36)-amide; Val^8Arg^{22}-GLP-1(7-36)-amide; Met^8Arg^{22}-GLP-1(7-36)-amide $Met^8Arg^{22}His^{37}$-GLP-1(7-36)-amide;Gly^8His^{22}-GLP-1(7-36)-amide; Val^8His^{22}-GLP-1(7-36)-amide; Met^8His^{22}-GLP-1(7-36-amide;His^{37}-GLP-1(7-36)-amide; $Val^8Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Arg^{22}His^{37}$-GLP-1(7-36)-amide; Gly^8His^{37}-GLP-1(7-36)-amide; Val^8His^{37}-GLP-

1(7-36)-amide; Met^8His^{37}-GLP-1(7-36)-amide;$Gly^8Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8Glu^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Glu^{22}His^{37}$-GLP-1(7-36)-amide;$Gly^8Lys^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8Lys^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Lys^{22}His^{37}$-GLP-1(7-36)-amide;$Gly^8Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8His^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8His^{22}His^{37}$-GLP-1(7-36)-amide; Val^8-GLP-1(7-37)OH, Gly^8-GLP-1(7-37)OH, Glu^{22}-GLP-1(7-37)OH, Asp^{22}-GLP-1(7-37)OH, Arg^{22}-GLP-1(7-37)OH, Lys^{22}-GLP-1(7-37)OH, Cys^{22}-GLP-1(7-37)OH, Val^8-Glu^{22}-GLP-1(7-37)OH, Val^8-Asp^{22}-GLP-1(7-37)OH, Val^8-Arg^{22}-GLP-1(7-37)OH, Val^8-Lys^{22}-GLP-1(7-37)OH, Val^8-Cys^{22}-GLP-1(7-37)OH, Gly^8-Glu^{22}-GLP-1(7-37)OH, Gly^8-Asp^{22}-GLP-1(7-37)OH, Gly^8-Arg^{22}-GLP-1(7-37)OH, Gly^8-Lys^{22}-GLP-1(7-37)OH, Gly^8-Cya^{22}-GLP-1(7-37)OH, Glu^{22}-GLP-1(7-36), NH_2, ASP^{22}-GLP-1(7-36)NH_2, Arg^{22}-GLP-1(7-36)NH_2, Lys^{22}-GLP-1(7-36)NH_2, Cys^{22}-GLP-1(7-36)NH_2, Val^8-Glu^{22}-GLP-1(7-36)NH_2, Val^8-Asp^{22}-GLP-1(7-36)NH_2, Val^8-Arg^{22}-GLP-1(7-36)NH_2, Val^8-Lys^{22}-GLP-1(7-36)NH_2, Val^8-Cys^{22}-GLP-1(7-36)NH_2, Gly^8-Glu^{22}-GLP-1(7-36)NH_2, Gly^8-Asp^{22}-GLP-1(7-36)NH_2, Gly^8-Arg^{22}-GLP-1(7-36)NH_2, Gly^8-Lys^{22}-GLP-1(7-36)NH_2, Gly^8-Cys^{22}-GLP-1(7-36)NH_2, Lys^{23}-GLP-1(7-37)OH, Val^8-Lys^{23}-GLP-1(7-37)OH, Gly^8-Lys^{23}-GLP-1(7-37)OH, His^{24}-GLP-1(7-37)OH, Val^8-His^{24}-GLP-1(7-37)OH, Gly^8-His^{24}-GLP-1(7-37)OH, Lys^{24}-GLP-1(7-37)OH, Val^8-Lys^{24}-GLP-1(7-37)OH, Gly8-Lys23-GLP-1(7-37)OH, Glu^{30}-GLP-1(7-37)OH, Val^8-Glu^{30}-GLP-1(7-37)OH, Gly^8-Glu^{30}-GLP-1(7-37)OH, Asp^{30}-GLP-1(7-37)OH, Val^8-Asp^{30}-GLP-1(7-37)OH, Gly^8-Asp^{30}-GLP-1(7-37)OH, Gln^{30}-GLP-1(7-37)OH, Val^8-Gln^{30}-GLP-1(7-37)OH, Gly^8-Gln^{30}GLP-1(7-37)OH, Tyr^{30}-GLP-1(7-37)OH, Val^8-Tyr^{30}-GLP-1(7-37)OH, Gly^8-Tyr^{30}-GLP-1(7-37)OH, Ser^{30}-GLP-1(7-37)OH, Val^8-Ser^{30}-GLP-1(7-37)OH, Gly^8-Ser^{30}-GLP-1(7-37)OH, His^{30}-GLP-1(7-37)OH, Val^8-His^{30}-GLP-1(7-37)OH, Gly^8-His^{30}-GLP-1(7-37)OH, Glu^{34}-GLP-1(7-37)OH, Val^8-Glu^{34}-GLP-1(7-37)OH, Gly^8-Glu^{34}-GLP-1(7-37)OH, Ala^{34}-GLP-1(7-37)OH, Val^8-Ala^{34}-GLP-1(7-37)OH, Gly^8-Ala^{34}-GLP-1(7-37)OH, Gly^{34}-GLP-1(7-37)OH, Val^8-Gly^{34}-GLP-1(7-37)OH, Gly^8-Gly^{34}-GLP-1(7-37)OH, Ala^{35}-GLP-1(7-37)OH, Val^8-Ala^{35}-GLP-1(7-37)OH, Gly^8-Ala^{35}-GLP-1(7-37)OH, Lys^{35}-GLP-1(7-37)OH, Val^8-Lys^{35}-GLP-1(7-37)OH, Gly^8-Lys^{35}-GLP-1(7-37)OH, His^{35}-GLP-1(7-37)OH Val^8-His^{35}-GLP-1(7-37)OH, Gly^8-His^{35}-GLP-1(7-37)OH, Pro^{35}-GLP-1(7-37)OH, Val^8-Pro^{35}-GLP-1(7-37)OH, Gly^8-Pro^{35}-GLP-1(7-37)OH, Glu^{35}-GLP-1(7-37)OH, Val^8-Glu^{35}-GLP-1(7-37)OH, Gly^8-Glu^{35}-GLP-1(7-37)OH, Val^8-Ala^{27}-GLP-1(7-37)OH, Val^8-His^{37}-GLP-1(7-37)OH, Val^8-Glu^{22}-Lys^{23}-GLP-1(7-37)OH, Val^8-Glu^{22}-Glu^{23}-GLP-1(7-37)OH, Val^8-Glu^{22}-Ala^{27}-GLP-1(7-37)OH, Val^8-Gly^{34}-Lys^{35}-GLP-1(7-37)OH, Val^8-His^{37}-GLP-1(7-37)OH, and Gly^8-His^{37}-GLP-1(7-37)OH, and derivatives thereof.

In a particular embodiment a GLP-1 agonist comprises or is selected from the group consisting of Val^8-GLP-1(7-37)OH, Gly^8-GLP-1(7-37)OH, Glu^{22}-GLP-1(7-37)OH, Lys^{22}-GLP-1(7-37)OH, Val^8-Glu^{22}-GLP-1(7-37)OH, Val^8- Lys^{22}-GLP-1(7-37)OH, Gly^8-Glu^{22}-GLP-1(7-37)OH, Gly^8-Lys^{22}-GLP-1(7-37)OH, Glu^{22}-GLP-1(7-36)NH_2, Lys^{22}-GLP-1(7-36)NH_2, Val^8-Glu^{22}-GLP-1(7-36)NH_2, Val^8-Lys^{22}-GLP-1(7-36)NH_2, Gly^8-Glu^{22}-GLP-1(7-36)NH_2, Gly^8-Lys^{22}-GLP-1(7-36)NH_2, Val^8-His^{37}-GLP-1(7-37)OH, Gly^8-His^{37}-GLP-1(7-37)OH, Arg^{34}-GLP-1(7-36)NH_2, and Arg^{34}-GLP-1(7-37)OH.

In another particular embodiment, the GLP-1 agonist comprises or is selected from the group consisting ofGly^8-GLP-1(7-37), Val^8GLP-1(7-37), Val^8Asp^{22}GLP-1(7-37), Val^8Glu^{22}GLP-1(7-37), Val^8Lys^{22}GLP-1(7-37), and Val^8His^{22} GLP-1(7-37), and analogs and derivatives thereof.

In yet another embodiment the GLP-1 agonist comprises or is selected from the group consisting of $Val^{8}Trp^{19}Glu^{22}$-GLP-1(7-37), $Val^{8}Glu^{22}Val^{25}$-GLP-1(7-37), $Val^{8}Tyr^{16}Glu^{22}$-GLP-1(7-37), $Val^{8}Trp^{16}Glu^{22}$-GLP-1(7-37), $Val^{8}Leu^{16}Glu^{22}$-GLP-1(7-37), $Val^{8}Tyr^{18}Glu^{22}$-GLP-1(7-37), $Val^{8}Glu^{22}His^{37}$-GLP-1(7-37), $Val^{8}Glu^{22}Ile^{33}$-GLP-1(7-37), $Val^{8}Trp^{16}Glu^{22}Val^{25}Ile^{33}$-GLP-1(7-37), $Val^{8}Trp^{16}Glu^{22}Ile^{33}$-GLP-1(7-37), $Val^{8}Glu^{22}Val^{25}Ile^{33}$-GLP-1(7-37), $Val^{8}Trp^{16}Glu^{22}Val^{25}$-GLP-1(7-37), analogues thereof and derivatives of any of these.

In yet another embodiment the GLP-1 agonist is a stable GLP-1 analogue/derivative. A "stable GLP-1 analogue/derivative" means a GLP-1 analogue or a derivative of a GLP-1 analogue which exhibits an *in vivo* plasma elimination half-life of at least 10 hours in man, as determined by the method described below. Examples of stable GLP-1 analogue/derivatives can be found in WO 98/08871 and WO 99/43706. The method for determination of plasma elimination half-life of a compound in man is summarized as follows. The compound is dissolved in an isotonic buffer, pH 7.4, PBS or any other suitable buffer. The dose is injected peripherally, preferably in the abdominal or upper thigh. Blood samples for determination of active compound are taken at frequent intervals, and for a sufficient duration to cover the terminal elimination part (e.g. Pre-dose, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 (day 2), 36 (day 2), 48 (day 3), 60 (day 3), 72 (day 4) and 84 (day 4) hours post dose). Determination of the concentration of active compound is performed as described in Wilken et al., Diabetologia 43(51):A143, 2000. Derived pharmacokinetic parameters are calculated from the concentration-time data for each individual subject by use of non-compartmental methods, using the commercially available software WinNonlin Version 2.1 (Pharsight, Cary, NC, USA). The terminal elimination rate constant is estimated by log-linear regression on the terminal log-linear part of the concentration-time curve, and used for calculating the elimination half-life.

Stable GLP-1 analogues and derivatives are disclosed in WO 98/08871 (analogues with lipophilic substituent) and in WO 02/46227 (analogues fused to serum albumin or to an Fc portion of an Ig).

In another embodiment, the GLP-1 agonist is formulated so as to have a half-life in man of at least 10 hours. This may be obtained by sustained release formulations known in the art.

Exemplary GLP-1 compositions include: BIM 51077 (GLP-1 analog resistant to DPP-IV digestion, available from Beautour Ipsen); AC2592 (GLP-1, from Amylin, San Ijiego CA); ThGLP-1 (GLP-1, modified amino acids and fatty acid attachment, from Theratechnologies, Saint-Laurent, Quebec, Canada); DAC:GLP-1 (Conjuchem, Montreal, Quebec, Canada); CJC-1131 or DAC_:GLP-1 (GLP-1 analog engineered for covalent coupling to albumin, Conjuchem), LY315902 and sustained release LY315902 (DDP-IV resistant GLP-1 analog from Eli Lilly, Indianapolis, IN); low molecular weight GLP-1 mimetic, Albugon (albumin: GLP-1 fusion peptide from Human Genome Sciences, Rockville, MD); Liraglutide or NN2211 (long acting GLP-1 derivative that is obtained by acylation of the GLP-1 molecule, which upon entering the bloodstream, is extensively bound to albumin which protects it from degradation by DPPIV and reduces renal clearance; Elbrond et al., Diabetes Care 2002 Aug 25(8): 1398-404).

In aspects of the invention, the GLP-1 agonist is an exendin including exendin agonists. An exendin includes naturally occurring exendin peptides that are found in the salivary secretions of the Gila-monster and the Mexican Bearded Lizard, reptiles that are endogenous to Arizona and Northern Mexico. An "exendin agonist" is a compound that fully or partially mimics or increases the effects, activity or reactions of an exendin, or reduces

or prevents inhibition of any effect, activity or reaction of an exendin. Exendins also include analogues and derivatives of a naturally occurring exendin peptide, in particular a stable analogue or derivative of a naturally occurring exendin peptide.

Exendins include without limitation exendin-4, exendin-3, or analogs or derivatives thereof. Exendin-3 is present in the salivary secretions of *Heloderma horridum* (Mexican Beaded Lizard) (Eng, J., et al., *J. Biol. Chem.*, 267:7402-05, 1992). Exendin-4 is a novel peptide from *Heloderma suspectum* (Gila monster) venom, having 53% homology with GLP-1 (7-36)amide (*J. Biol. Chem.*, 267:7402-05, 1992). Sequences of exendin-4 peptides are in SEQ ID NOs. 23 through 35. Exendin-4 functions as a long-acting potent agonist of the glucagon-like peptide 1 (GLP-1) receptor, as it is resistant to degradation by DDP-IV. Exendin-4 has properties similar to GLP-1, and regulates gastric emptying, insulin secretion, food intake, and glucagon secretion. Examples of exendin-4 include exenatide (synthetic form also known as AC2993 or Byetta™, Amylin); exenatide LAR (long acting form); ZP10 (modified exendin 4 having addition of six lysine residues, Aventis/Zealand Pharma), and AP10 (long acting formulation, Alkermes, Cambridge MA). Physiological studies indicate that sustained expression of exendin-4 in transgenic mammals does not perturb glucose homeostasis, cell mass or food intake (Biaggio, L. et al. J Biol Chem 275: 34472-34477, 2000), so that the physiological effects of exendin-4 are not completely understood.

In embodiments of the invention, the exendin is exendin-3. In other embodiments, the exendin is exendin-4, in particular exenatide, more particularly Byetta™.

Examples of analogues and derivatives include an exendin-4(1-39) analogue or a derivative of an exendin-4(1-39).

Examples of exendins as well as analogues, derivatives, fragments, and agonists thereof are disclosed in WO 97/46584, US Pat. No. 5,424,286, WO 01/04156, US Pat. Nos. 6,956,026, 6,924,264, 6,902,744, 6,528,486, and 6,506,724 and references therein. US 5,424,286 describes a method for stimulating insulin release with an exendin polypeptide. The exendin polypeptides disclosed include HGEGTFTSDLSKQMEEEAVRLFIEWLK NGGX [SEQ ID NO. 27] wherein X = P or Y, and HX1X2GTFITSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS [SEQ ID NO. 21]; wherein X1X2 = SD (exendin-3) or GE (exendin-4)). WO 97/46584 describes truncated versions of exendin peptide(s). The disclosed peptides increase secretion and biosynthesis of insulin, but reduce those of glucagon. WO 01/04156 describes exendin-4 analogues and derivatives as well as the preparation of these molecules. Exendin-4 analogues stabilized by fusion to serum albumin or Fc portion of an Ig are disclosed in WO 02/46227.

In embodiments, an exendin has the empirical formula $C_{184}H_{282}N_{50}O_{60}S$ and a molecular weight of 41.86.6 daltons, and the following amino acid sequence: H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2 [SEQ ID NO. 24]. In an embodiment, the exendin agonist has an amino acid sequence comprising His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser [SEQ ID NO.23].

Particular exendins include the following: [SEQ ID NO 25: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly], exendin-4 (1-30) amide [SEQ ID NO 26: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe

Ile Glu Trp Leu Lys Asn Gly Gly-NH_2], exendin-4 (1-28) amide [SEQ ID NO 31: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH_2], ^{14}Leu, ^{25}Phe exendin-4 amide [SEQ ID NO 32: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH_2], ^{14}Leu, ^{25}Phe exendin-4 (1-28) amide [SEQ ID NO 33: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH_2], ^{14}Leu, ^{22}Ala, ^{25}Phe exendin-4 (1-28) amide [SEQ ID NO 34: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Ala Ile Glu Phe Leu Lys Asn-NH_2], and His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Ser Lys Lys Lys Lys Lys Lys [SEQ ID NO. 30].

Aspects of the invention may utilize an exendin precursor, in particular Met-Lys-Ile-Ile-Leu-Trp-Leu-Cys-Val-Phe--Gly-Leu-Phe-Leu-Ala-Thr-Leu-Phe-Pro-Ile--Ser-Trp-Gln-Met-Pro-Val-Glu-Ser-Gly-Leu--Ser-Ser-Glu-Asp-Ser-Ala-Ser-Ser-Glu-Ser--Phe-Ala-Ser-Lys-Ile-Lys-Arg-His-Gly-Glu--Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln---Asn-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile--Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser--Gly-Ala-Pro-Pro-Pro-Ser-Gly [SEQ ID NO. 35].

In a preferred aspect, the exendin comprises exendin-4. In other preferred aspects, the pharmaceutical composition comprises a peptide selected from: exendin-4 (1-30), exendin-4 (1-30) amide, exendin-4 (1-28) amide, ^{14}Leu,^{25}Phe exendin-4 amide, ^{14}Leu,^{25}Phe exendin-4 (1-28) amide, and ^{14}Leu,^{22}Ala, ^{25}Phe exendin-4 (1-28) amide.

Examples of exendin analogs include without limitation exendin agonist compounds such as those described in U.S. Provisional Application US20060035836A1, US20050267034A1, US6989366, US6956026, and publications and patents and patent applications referenced therein.

In yet another embodiment the GLP-1 agonist is a stable exendin-4 analogue/derivative. The term "stable exendin-4 analogue/derivative", as used herein refers to an exendin-4(1-39) analogue or a derivative of an exendin-4(1-39) analogue which exhibits an *in vivo* plasma elimination half-life of at least 10 hours in man, as determined by the method described above for a "stable GLP-1 analogue/derivative".

In still another embodiment, the GLP-1 agonist is $Aib^{8,35}$ GLP-1(7-36) amide (Aib = a-amino isobutyric acid).

In still another embodiment, the GLP-1 agonist is Ser^{38},$Lys^{39,40,41,42,43,44}$-Exendin-4(1-39)amide.

In still another embodiment the GLP-1 agonist is selected from the non-peptide small molecule GLP-1 agonists disclosed in WO 00/42026.

An amino acid portion of a GLP-1 agonist can be prepared by a variety of methods known in the art such as solid-phase synthesis, purification of GLP-1 agonists from natural sources, recombinant technology, or a combination of these methods. See for example, United States Patent Nos. 5,188,666, 5,120,712, 5,523,549, 5,512,549, 5,977,071, 6,191,102, Dugas and Penney 1981, Merrifield, 1962, Stewart and Young 1969, and the references cited herein. GLP-1 agonist derivatives can be produced by appropriate derivatization of an appropriate backbone produced, for example, by recombinant DNA technology or peptide synthesis (e.g. Merrifield-type solid phase synthesis) using methods known in the art of peptide synthesis and peptide chemistry.

A "GLP-1 agonist" includes substances that decrease inactivation or increase activity, function or reactions of glucagon-like peptide 1 receptor ligands. Examples of such substances include DPP IV inhibitors. GLP-1 agonists may be produced by methods known in the art and they may be administered at therapeutically effective doses known in the art for the compounds.

In the present context a "DPP IV inhibitor" is an antagonist of a dipeptidylpeptidase-IV (DPP IV) or another member of the family of serine peptidases that includes quiescent cell proline dipeptidase, DPP8, and DPP9. It exhibits inhibition of the enzymatic activity of DPP IV and functionally related enzymes, such as from 1-100% inhibition, and especially preserves the action of substrate molecules, including but not limited to GLP-1, GIP, peptide histidine methionine, and other similar molecules,. A DPP IV inhibitor may indirectly affect the levels of GLP-1 (Hughes, T. et al., 2002, Am I Diabetes Assoc Abstract 272) by inhibiting an enzyme involved in its integrity. A DPP-IV inhibitor can be peptidic or non-peptidic, in particular the DPP- IV inhibitor is non-peptidic.

The term "DPP IV inhibitor" is also intended to comprise active metabolites and prodrugs of a DPP-IV inhibitor, such as active metabolites and prodrugs of DPP-IV inhibitors. A "metabolite" refers to an active derivative of a DPP-IV inhibitor produced when the DPP-IV inhibitor is metabolized. A "prodrug" of a DPP-IV inhibitor refers to a compound that is either metabolized to a DPP-IV inhibitor or is metabolized to the same metabolite(s) as a DPP-IV inhibitor. The inhibitors also include the corresponding stereoisomers as well as the corresponding polymorphs, e.g., crystal modifications, which are disclosed in the cited patent documents.

Representative DPP-IV inhibitors are listed in Table 2 and in WO 05049088 and references therein. DPP-IV inhibitors are in each case generically and specifically disclosed in the referenced patent document or publication. Any of the substances disclosed in the patent documents and publications referenced in Table 2 are considered potentially useful as DPP-IV inhibitors to be used in carrying out the present invention.

In particular aspects of the invention, the DPP-IV inhibitor is sitagliptin, vildagliptin, PSN9301, saxagliptin, N-(N'-substituted glycyl)-2-cyanopyrrolidines, L-threo-isoleucyl thiazolidine (P32/98), L-allo-isoleucyl thiazolidine, L-threo-isoleucyl pyrrolidine, isoleucine thiazolidide, valine purrolidide, NVP-DPP738 (Novartis, Cambridge, MA), LAP237 (Novartis), P32/98 (Probiodrug AG, Halle, Germany), P93/01 (Probiodrug), and L-allo-isoleucyl pyrrolidine described in U.S. Pat. No. 6,001,155, WO 99/61431, WO 99/67278, WO 99/67279, DE 198 34 591, WO 97/40832, DE 196 16 486 C_2, WO 98/19998, WO 00/07617, WO 99/38501, and WO 99/46272, and US20050222221. A DPP IV inhibitor can be prepared by a variety of methods known in the art.

Particular DPP-IV inhibitors for the combinations, uses, methods, and kits of the present invention are 1-{2-[(5-cyanopyridin-2-yl) amino]ethylamino}acetyl-2 (S)- cyano-pyrrolidine dihydrochloride (DPP728), especially the dihydrochloride thereof; (S)-1-[(3- hydroxy-1-adamantyl)amino]acetyl-2-cyano-pyrrolidine (LAF237); L-threo-isoleucyl thiazolidine (compound code according to Probiodrug: P32/98); MK-0431; GSK23A; BMS-477118; 3-(aminomethyl)-2-isobuthyl-1-oxo-4-phenyl-1,2-dihydro-6-isoquinoline carboxamide and 2-{[3-(aminomethyl)-2-isobuthyl-4-phenyl-1-oxo-1,2-dihydro-6- isoquinolyl]oxy} acetamide and optionally in any case pharmaceutical salts thereof.

The term, "prolactin" as used herein means any polypeptide which shares substantial sequence identity or similarity with an endogenous mammalian prolactin as this term is known in the art of protein factors, for

example, human prolactin, and which possesses the activity of a prolactin. Endogenous human prolactin is a 199 amino acid polypeptide produced by the pituitary gland. The term encompasses prolactin analogs which are deletions, insertions, or substitution mutants of endogenous prolactin, and retain the activity, and includes prolactins from other species and naturally occurring variants. The prolactin function includes a, composition having agonist activity for the prolactin receptor, as disclosed in U.S. Patent No. 6,333.031 (activating amino acid sequence) and No. 6,413,952 (metal complexed receptor 5 ligand agonist), and G120RhGH, which is an analog of human growth hormone that acts as a prolactin agonist (Mode et al., 1966, Endoerinol. 137(2): 447-454), and a ligand for the prolactin receptor as described in U.S. Patent Nos. 5,506,107 and 5,837,460.

PRL, GH and PL are members of a family of polypeptide hormones that share structural, immunological and biological functions (reviewed in, "Pancreatic Growth and Regeneration", Ed. N. Sarvetniek, Ch. 1. Brejie, T. et al., 1997), and therefore are referred to herein as the PRL/GH/PL family. PRL and GH are secreted by the anterior pituitary of vertebrate animals. PRL is involved in a broad range of biological functions that include osmoregulation, reproduction, lactation, and immunomodulation. GH is associated with physiological processes related to growth and morphogenesis. The related receptor ligands are referred to as "PRL/GH/PL" receptor ligands.

A growth/hormone regulatory factor that is a "growth hormone" encompasses any polypeptide that shares substantial amino acid sequence identity with an endogenous mammalian growth hormone and possesses a biological activity of a mammalian growth hormone. Human growth hormone is a polypeptide containing 191 amino acids in a single chain, and a molecular weight of about 22 kDal (Goeddel et al., 1979, Nature 281: 544-548; Gray et al., 1985, Gene 39: 47-254). The term encompasses analogs having deletions, insertions or substitutions and growth hormones from other species and naturally occurring variants. See Cunningham et al., 1989, Science 243: 1330-1336, and 1989, Science 244: 1081-1085; and WO 90/05185, and U.S. Pat. No. 5,506,107.

The term, "erythropoietin" (EPO) as used herein is any endogenous mammalian EPO or variant thereof, or EPO receptor agonist, for example the EPO mimetic EMP1 (Johnson et al., 2000, Nephr Dial Tranpl 15:1274-1277); or mimetics described, for example, in Wrighton et al., 1996, Science 273:458-464; U.S. patent number 5,773,569; Kaushansky, 2001, Ann NY Acad Sci lo 938: 131 - 138; an antibody having EPO receptor agonist activity (see for example, U.S. patent number 5,885,574; WO 96/40231); and an amino acid sequence disclosed in U.S. Patent Numbers 6,333,031, and 6,413,952.

The term, "PACAP" as used herein means an endogenously produced PACAP or analog or variant thereof that shares substantial amino acid sequence identity or similarity, or has 15 biological activity as a PACAP receptor agonist such as maxadilan (Moro et al., 1997, J Biol Chem 272:966-970. Useful PACAP variants include without limitation, the 38 amino acid and 27 amino acid variants disclosed in U.S. Patent Numbers 5,128,242; 5,198, 542; 5,208.320; and 6,242,563).

A "gastrin/CCK receptor" refers to a member of the G-protein-coupled receptor family that displays a characteristic binding affinity for a cholecystokinin (CCK) including without limitation CCK-8, desulfated CCK-8, CCK-33, CCK-4, or gastrins including without limitation desulfated or sulfated gastrin-17, or pentagastrin, or other CCK or gastrin analogues or family members. Examples of CCK/gastrin receptor proteins are CCK_A and CCK_B/gastrin receptors, in particular CCK_B/gastrin receptor.

A "gastrin compound" refers to any compound, including peptides and non-peptide compounds, which fully or partially associate with and/or activate a gastrin/CCK receptor and/or increase gastrin secretion. In aspects of the invention, a gastrin compound is selected that has a suitable IC_{50}, for example an IC_{50} of about ~ 0.7 nM at a gastrin/CCK receptor, as measured by methods known in the art (see Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin et al (1995) J. Biol. Chem. 270: 5019-5023 describing *in vitro* cell growth assays, and receptor binding assays as described in Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin et al (1995) J. Biol. Chem. 270: 5019-5023). A gastrin compound may also be selected based on other criteria such as activity, half-life etc.

Gastrin compounds that may be used in the present invention include without limitation one or more of a gastrin compound including a cholecystokinin, or a cholecytokinin agonist.

The term "gastrin compound" encompasses compounds that in combination with a growth/hormone regulatory factor and a gastrin agonist provide at least one beneficial effect. In other aspects of the invention a gastrin compound is selected to stimulate a growth/hormone regulatory factor such that when a gastrin compound in the same or adjacent tissue or in the same individual is also stimulated, neogenesis of insulin-producing pancreatic islet cells is induced. In a further aspect the term includes any gastrin compound that demonstrates additive, synergistic, or complementary activity with a growth/hormone regulatory factor and a gastrin agonist.

The term includes analogs, derivatives, fragments and modifications of a wild-type gastrin and chimeric polypeptides comprising gastrin. In aspects of the invention a gastrin compound includes a polypeptide that shares substantial amino acid sequence identity with a mammalian gastrin and possesses some or all of the biological activity of a mammalian gastrin. In certain aspects, a gastrin compound may be an active analog, fragment or other modification which, for example, share amino acid sequence with an endogenous mammalian gastrin, for example, share 60% sequence identity, or 70% sequence identity, or 80% sequence identity

A "gastrin compound" includes, without limitation, the various forms of gastrin, such as gastrin 71, gastrin 52, gastrin 34 (big gastrin), gastrin 17 (little gastrin), gastrin 14, and gastrin 8 (mini gastrin), pentagastrin, tetragastrin, and fragments, analogs, and derivatives thereof. Sequences for gastrins including big gastrin-34 (Bonato et al, 1986, Life Science 39:959) and small gastrin-17 (Bentley et al (1966) Nature 209:583) are known in the art, and some are shown in SEQ ID NOs. 1 to 9. In particular, sequences for gastrins include gastrin 71 of SEQ ID NO. 5 (residues 22-92), gastrin 52 of SEQ ID NO. 6, gastrin 34 (big gastrin) of SEQ ID NOs. 1 or 2, gastrin 17 (little gastrin) of SEQ ID NO. 3 or 4, gastrin 14 of SEQ ID NO. 7, and gastrin 6 of SEQ ID NO.8 or 9. Gastrin-34 is essentially an extension of an amino acid sequence at the N-terminal end of gastrin-17. Big gastrin is cleaved *in vivo* to release gastrin-17. Glp at the N-terminal end of a gastrin is pyroglutamate, which is a naturally cyclized form of glutamate. In various embodiments, where cysteine or lysine is added to a terminus of gastrin having a pyroglutamate, the pyroglutamate is replaced with a glutamate, or the pyroglutamate is deleted. A gastrin 34 or gastrin-17 may be used in the invention where there is a methionine or a leucine at position 15, as shown in SEQ ID NOs: 1-4 herein.

Examples of gastrin compounds that may be used in the present invention include the compounds disclosed in U.S. Patent No. 6,288,301. In some applications of the invention, a gastrin compound may be selected that is a peptide or non-peptide agonist or partial agonist of the gastrin receptor such as A71378 (Lin et al., Am. J. Physiol. 258 (4 Pt 1): G648, 1990).

In some applications of the invention, a gastrin compound may be selected that is a gastrin/CCK_B receptor ligand including but not limited to cholecystokinin (CCK) such as CCK 58, CCK 33, CCK 22, CCK 12 and CCK 8; and the like.

A "gastrin compound" includes a modified form of a gastrin, including but not limited to a modified form of gastrin 71 [SEQ ID NO. 5, residues 22 to 92], gastrin 52 [SEQ ID NO. 6], gastrin 34 (big gastrin) [SEQ ID NO. 1 or 2], gastrin 17 (little gastrin) [SEQ ID NO. 3 or 4], gastrin 14 [SEQ ID NO. 7], gastrin 8, gastrin 6 [SEQ ID NO.8 or 9], pentagastrin, and tetragastrin. A modified gastrin preferably comprises TrpMetAspPhe-NH_2 [SEQ ID NO. 13] or TrpLeuAspPhe-NH_2 [SEQ ID NO.14].

In aspects of the invention a modified gastrin comprises at least amino acids 1-34, 18-34 or 29-34 of SEQ ID NO. 1 or 2, or amino acids 1-17, 2-17, 12-17, or 14-17 of SEQ ID NO. 3 or 4.

A gastrin compound used in aspects of the methods, compositions, and conjugates of the invention may comprise gastrin 17 and analogs and derivatives thereof. In particular aspects, the gastrin compound is synthetic human gastrin 1 having 17 amino acid residues with a Leu residue at amino acid position 15 [SEQ ID NO. 4].

A gastrin compound used in the methods, compositions and conjugates of the invention may comprise gastrin 34 and analogs and derivatives thereof. In particular aspects, the gastrin compound is a synthetic human gastrin 34 with methionine or leucine at position 32 [SEQ ID NO. 1 or 2].

Modified gastrin compounds for use in the present invention comprise the modified gastrin compounds described in PCT/CA03/01778 and US Serial No. 10/728,082 incorporated in their entirety by reference.

In particular, a modified gastrin can be a gastrin derivative or analogue comprising a minimal sequence of 6 amino acids (from the C-terminal end) of a gastrin, in particular amino acid residues 1 to 34, 18 to 34 or 29-34 of SEQ ID NO: 1 or 2, or amino acid residues 1-17, 2-17, 12-17, or 14-17 of SEQ ID NO. 3 or 4, and comprising a reactive group capable of undergoing an addition reaction. Examples of reactive groups include without limitation thiols, alpha amino groups, epsilon amino groups, carboxyl groups or aromatic rings. A reactive group is generally capable of linking a gastrin sequence, directly or indirectly via a crosslinking agent and/or spacer region, to a carrier.

A reactive group may be introduced by adding or substituting an amino acid comprising a reactive group, for example by adding a cysteine or lysine. Therefore, a modified gastrin may comprise a gastrin sequence (e.g. gastrin-34 or gastrin 17) wherein at least one reactive amino acid (e.g. cysteine or lysine) is added or substituted. The addition of a reactive amino acid can be at a terminal region, in particular an N-terminal region.

A modified gastrin may also optionally comprise a spacer. A spacer can interact with a reactive group, for example, an amino acid comprising a reactive group. A spacer can be one or more amino acids, peptides, peptidomimetics, or small organic molecules. A spacer can comprise at least one amino acid, preferably at least two, three, four or five amino acids and in certain embodiments it is a sequence of several amino acids, including without limitation alanine or glycine. A spacer can comprise alternating amino acids (e.g. glycine and/or alanine), non-alternating amino acids, a random sequence or a particular sequence. By way of example, a spacer can be synthesized as part of, or may be chemically attached to an amino acid of a gastrin sequence.

A modified gastrin may optionally comprise a cross-linking agent. A cross-linking agent may comprise a homobifunctional or heterobifunctional portion for interaction directly or indirectly with a gastrin, spacer

and/or a reactive group. A cross-linking agent may interact with a gastrin sequence or a spacer, or it may be added to a reactive group at the end (in particular N-terminus) of a modified gastrin.

A cross-linking agent can be any agent that can link a gastrin sequence and a carrier directly or via a spacer. Examples of homobifunctional crosslinking agents include without limitation amino group directed homobifunctional cross-linking reagents such as bisimidates (e.g. methyl acetimidate-HCl), bifunctional aryl halides (e.g. 1,5-dichloro-2,4-dinitrobenzene), bifunctional acylating agents (e.g. diisocyanates), bifunctional sulfonyl halides (e.g. phenol-2,4-disulfonyl-chloride), bifunctional acylazides (e.g. tartryl diazide), dialdehydes (e.g. glutaraldehyde), and diketones (e.g. 2,5-hexanedione). Examples of heterobifunctional crosslinkers include amino and sulfhydryl group directed bifunctional reagents (e.g. N-succinimidyl-3-(2-pyridyldithio propionate, carboxyl and either sulfhydryl or amino group directed bifunctional reagents (e.g. p-nitrophenyl diazoacetate), and carbonyl and sulfhydryl group directed bifunctional reagents (e.g. 1-(aminooxy)-4-[3-nitro-2-pyridyl)dithio)]butane).

A modified gastrin can optionally comprise a carrier which may be a polymer. A carrier may be a polymer of amino acids (proteins), sugars (polysaccharides), nucleosides, synthetic polymers and mixtures thereof. A protein carrier may be a protein found in the circulatory system. Examples of protein carriers found in the circulatory system, in particular the human circulatory system, include without limitation plasma components such as serum, purified serum proteins such as albumin (in particular human serum albumin), transferrin, or an immunoglobulin, red blood cell proteins such as glycophorin A and AE-1, sugar binding proteins such as a lectin, inactivated enzymes, phosphate and sulphate binding proteins, and lipid binding proteins. Examples of other suitable polymeric carriers include without limitation cellulose and derivatives thereof, starch and derivatives thereof, heparin and derivatives thereof, and synthetic polymers such as polyethylene glycol (PEG) and dextran, and derivatives thereof. Carriers may be attached to a gastrin or spacer by way of reactive groups on, or introduced to, the carrier, gastrin, and/or spacer. For example, carriers can be covalently attached to reactive groups (such as thiol groups, alpha and epsilon amino groups, carboxyl groups or aromatic groups) on a gastrin or spacer which may be present or added by chemical modification of the gastrin or spacer.

In certain aspects of the invention, a modified gastrin can comprise a gastrin of SEQ ID NOS 1, 2, 3, 4, 7, or 8 and a carrier.

A group of modified gastrin compounds include compounds having an amino acid sequence comprising from the amino terminus $Z\text{-}Y_m\text{-}X_n\text{-}AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6$, wherein AA_1 is Tyr or Phe, AA_2 is Gly, Ala, or Ser, AA_3 is Trp, Val, or Ile, AA_4 is Met or Leu, AA_5 is Asp or Glu, and AA_6 is Phe or Tyr and wherein AA_6 is optionally amidated; Z is a carrier, in particular a polymer and when the polymer is a protein Z is an amino acid sequence; Y_m is an optional spacer region comprising *m* amino acid residues of a small neutral amino acid including but not limited to serine and alanine, and X is any consecutive portion of residues 1-28 (=n) of SEQ ID NO: 1 or 2 or 1-11 of SEQ ID. NO. 3 or 4 providing that the gastrin compound binds a gastrin/CCK_B receptor. Generally, *m* is 0 to about 20 residues. In an aspect Z is a protein, in particular a protein of the circulatory system, more particularly a serum protein, still more particularly albumin, most particularly human serum albumin.

In embodiments, X is one or more amino acid residues from position 18 to position 28 of SEQ ID NO: 1. Therefore, the gastrin compounds by virtue of the presence of X can have any of gastrin sequences from

positions 18-28, 19-28, 20-28, 21-28, etc. The gastrin compound optionally contains an amino acid spacer (Y) of length *m*, and *m* is 0 to about 20 residues.

In embodiments, X is one or more amino acid residues from position 1 to 11 or 2 to 11 of SEQ ID NO: 3 or 4. Therefore, the gastrin compounds by virtue of the presence of X can have any of gastrin sequences from positions 2 to 11, 3 to 11, 4 to 11, 5 to 11, etc. The gastrin compound optionally contains an amino acid spacer (Y) of length *m*, and *m* is 0 to about 20 residues.

A gastrin compound includes a modified gastrin compound of the formula X_n-AA_1-AA_2-AA_3-AA_4-AA_5-AA_6, where there is no spacer (Y) and *m* is 0, which may further comprise a bifunctional cross-linking agent for interaction or linkage to a carrier Z, where Z further comprises a non-proteinaceous polymer such as dextran or PEG.

A modified gastrin compound particularly described herein may further comprise an amino terminal cysteine or lysine residue.

In some embodiments of modified gastrin compounds described herein, the gastrin component contains at least amino acid residues 29-34 of SEQ ID NO: 1 or 2, and it is associated with a polymer, a lipid or a carbohydrate. The polymer may be a synthetic or naturally occurring polymer. The term polymer includes a protein polymer of amino acids, and is not limited to a synthetic polymer. The polymer may be a polyethylene glycol (PEG) or a dextran. A modified gastrin compound can be based on SEQ ID NO: 1 or 2 or "big" gastrin-34 and have a residue at position 32 which is a methionine or a leucine, respectively.

Another preferred modified gastrin compound comprises a structure C-Y_m-X, wherein C is Cys or Lys, Y_m is an optional spacer region comprising m amino acid residues of a small neutral amino acid, and X is at least six amino acid residues comprising at least positions 12-17 of gastrin-17 (SEQ ID NO: 3 or 4) or at least positions 29-34 of gastrin-34 (SEQ ID NO: 1 or 2). This modified gastrin compound can further comprise a bifunctional cross-linking agent wherein one reactive portion of the cross-linking agent is covalently linked to C, and the other reactive portion is covalently linked to a polymer or protein.

In a particular aspect of the invention AA_1-AA_2-AA_3-AA_4-AA_5-AA_6 in a modified gastrin compound is Tyr-Gly-Trp-Met-Asp-Phe [SEQ ID NO. 10] or Tyr-Gly-Trp-Leu-Asp-Phe [SEQ ID NO.11].

In a further aspect of the invention, a gastrin compound used in the methods, compositions and conjugates of the invention is gastrin 34 or gastrin 17 or portions thereof, directly or indirectly interacting or associated with a serum protein, in particular albumin or an immunoglobulin, more particularly human serum albumin.

In aspects of the invention, a gastrin compound comprises synthetic human gastrin 34 having 2-34 amino acid residues of SEQ ID NO. 1 or 2, and optionally an N-terminal cysteine and/or a carrier; synthetic human gastrin having 1-17 amino acid residues with a Leu residue at amino acid position 15 [SEQ ID NO. 4] and optionally an N-terminal cysteine residue; and a synthetic human gastrin having amino acid residues 2 to 17 or 5-17 of SEQ ID NO. 3 or 4, optionally with an N-terminal cysteine residue and/or a carrier (e.g. PEG or human serum albumin) linked via a spacer [e.g. Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala i.e. $(GA)_5$] [SEQ ID NO. 12], in particular, a synthetic human gastrin having amino acid residues 2 to 17 or 5-17 of SEQ ID NO. 3 or 4, with a human serum albumin (HSA) polymer linked via a Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala [i.e., $(GA)_5$] spacer, and optionally an N-terminal cysteine residue.

Gastrin compounds may be synthesized by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, J. Am. Chem. Assoc. 85:2149-2154) or synthesis in homogenous solution (Houbenweyl, 1987, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart). The synthesis may be performed using manual procedures or by automation. Automated synthesis may be carried out, for example, using an Applied Biosystems 431A peptide synthesizer (Perkin Elmer). Gastrin compounds may also be obtained from commercial sources. For example, synthetic human gastrin 17 with methionine or leucine at position 15 are available from Bachem AG, Bubendorf, (Switzerland), and from Research Plus Inc (New Jersey, USA).

In particular aspects of the invention the gastrin compound is a leucine substituted gastrin 17 of SEQ ID NO. 3. Such a gastrin compound may also be characterized by the following properties: isoelectric point of about 3.4; purity of at least about 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, and/or a molecular mass of about 2080.2 ±2 Da.

An "immunosuppressive agent" refers to any agent which inhibits or prevents an immune response. Examples of immunosuppressive agents are listed in Table 3. Immunosuppressive agents are in each case generically and specifically disclosed in the referenced documents or publication. Any of the substances disclosed in the documents and publications referenced in Table 3 are considered potentially useful as immunosuppressive agents to be used in carrying out the present invention. The agents may be produced by methods known in the art and they may be administered at therapeutically effective doses known in the art for the compounds.

An exemplary immunosuppressive agent is a drug, for example, a rapamycin; a corticosteroid; an azathioprine; mycophenolate mofetil; a cyclosporine; a cyclophosphamide; a methotrexate; a 6-mercaptopurine; FK506; 15-deoxyspergualin; an FTY 720; a mitoxantrone; a 2-amino-1,3-propanediol; a 2-amino-2[2-(4-octylphenyl)ethyl]; propane-1,3-diol hydrochloride; a 6-(3 dimethyl-aminopropionyl) forskolin; and a demethimmunomycin. Alternatively, an immunosuppressive agent is a protein, for example, a protein comprising an amino acid sequence of an antibody. Accordingly, the immunosuppressive agent is at least one of: hut 124; BTI-322, allotrap-HLA 15 B270; OKT4A; Enlimomab; ABX-CBL; OKT3; ATGAM; basiliximab; daclizumab; thymoglobulin; ISAtx247; Medi-500; Medi-507; Alefacept; efalizumab; infliximab; and an interferon.

In aspects of the invention the agent is dexamethasone, cyclosporin A, azathioprine, brequinar, gusperimus, 6-mercaptopurine, mizoribine, rapamycin, tacrolimus (FK-506), folic acid analogs (e.g., denopterin, edatrexate, methotrexate, piritrexim, pteropterin, Tomudex®, trimetrexate), purine analogs (e.g., cladribine, fludarabine, 6-mercaptopurine, thiamiprine, thiaguanine), pyrimidine analogs (e.g., ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, doxifluridine, emitefur, enocitabine, floxuridine, fluorouracil, gemcitabine, tegafur), fluocinolone, triaminolone, anecortave acetate, flurometholone, medrysone, and prednislone.

An "insulin sensitivity enhancer" or "insulin resistance deblocker" refers to a substance that restores impaired insulin receptor function to deblock insulin resistance thereby enhancing insulin sensitivity. Exemplary insulin sensitivity enhancers are pioglitazone [Fujita et al., Diabetes, 32, 804-810, 1983, JP-A S55(1980)-22636 (EP-A 0008203), JP-A S61(1986)-267580 (EP-A 193256)], CS-045, PPAR(α) antagonists (fibrates), rexinoids, protein tyrosine kinase inhibitors β_3 adrenergic receptor antagonists, thiazolidinedione derivatives and substituted thiazolidinedione derivatives which may be used in combination with insulin [JP-A H4(1992)-66579, JP-A

H4(1992)-69383, JP-A H5(1993)-202042]. Insulin sensitivity enhancers may be produced by methods known in the art and they may be administered at therapeutically effective doses known in the art for the compounds.

In aspects of the invention insulin sensitivity enhancers include 5-[[3,4-dihydro-2-(phenylmethyl)-2H-1-benzopyran-6-yl]methyl]-2,4-thiazolidinedione (generic name: englitazone) or its sodium salt; 5-[[4-[3-(5-methyl-2-phenyl-4-oxazolyl)-1-oxopropyl]phenyl]methyl]-2,4-thiazalidinedione (generic name: darglitazone/CP-86325) or its sodium salt; 5-[2-(5-methyl-2-phenyl-4oxazolylmethyl)benzofuran-5ylmethyl]-2,4-oxazolidinedione(CP-92768); 5-(2-naphthalenylsulfonyl)-2,4-thiazolidinedione (AY-31637); 4-[(2-naphthalenyl)methyl]-3H-1,2,3,5-oxathiadiazol-2-oxide (AY-30711); and 5-[[4-[2-(methyl-2-pyridylamino)ethoxy]phenyl]-methyl]-2,4-thiazolidinedione(BRL-49653). Certain thiazolidinedione insulin sensitisers are also disclosed in European Patent Applications, Publication Numbers: 0008203, 0139421, 0032128, 0428312, 0489663, 0155845, 0257781, 0208420, 0177353, 0319189, 0332331, 0332332, 0528734, 0508740; and International Patent Application, Publication Numbers 92/18501, 93/02079, 93/22445 and U.S. Pat. Nos. 5,104,888 and 5,478,852.

In aspects of the invention an insulin sensitivity enhancer is a thiazolidinedione insulin sensitiser, in particular a thiazolidinedione insulin sensitiser including compounds comprising a 2,4-thiazolidinedione moiety. In embodiments of the invention the insulin sensitiser is a thiazolidinedione insulin sensitiser including without limitation (+)-5-[[4-[(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)methoxy]phenyl]methyl]-2,4-thiazolidine dione (or troglitazone), 5-[4-[(1-methylcyclohexvl)methoxy]benzyl]thiazolidine-2,4-dione (or ciglitazone), 5-[4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl]thiazolidine-2,4-dione (or pioglitazone) or 5-[(2-benzyl-2,3-dihydrobenzopyran)-5-ylmethyl)thiazolidine-2,4-dione (or englitazone), more particularly pioglitazone and rosiglitazone (Actos® and Avandia®).

Acyclic insulin sensitisers which have insulin sensitiser activity may also be utilized in the present invention. These sensitisers are illustrated in International Patent Applications, Publication Numbers WO93/21166 and WO94/01420, and in U.S. Pat. No. 5,232,945 and International Patent Applications, Publication Numbers WO92/03425 and WO91/19702. Other exemplary insulin sensitisers are those disclosed in European Patent Application, Publication Number 0533933, Japanese Patent Application Publication Number 05271204 and U.S. Pat. No. 5,264,451.

A "glucose lowering agent" refers to a substance having one or more of the following actions: stimulation of anaerobic glycolysis, increase of the sensitivity to insulin in the peripheral tissues, inhibition of glucose absorption from the intestine, suppression of hepatic gluconeogenesis, and inhibition of fatty acid oxidation. Glucose lowering agents may be produced by methods known in the art and they may be administered at therapeutically effective doses known in the art for the compounds.

Glucose lowering agents that may be used in the present invention include biguanide compounds, thiazolidinediones, and α-glucosidase inhibitors. Biguanide compounds include but are not limited to N-dimethylbiguanides, substituted or otherwise, and for example metformin, but also other pharmaceutical compounds, for example buformin or fenformin, or a salt thereof with a therapeutically compatible mineral acid or organic acid. In aspects of the invention, a glucose lowering agent is metformin hydrochloride. Metformin is commercially available in 500 mg, 850 mg and 1000 mg tablets under the GLUCOPHAGE® tradename from

Bristol Meyers Squibb. Metformin hydrochloride may be administered in humans at an initial daily dose of from 500 mg to about 800 mg and increased, as needed, to a maximum daily dosage of 2550 mg.

α-glucosidase inhibitors, including without limitation acarbose (Precose®) and miglitol (Glycet®), inhibit α-glucosidase enzymes resulting in the reduction of glucose concentrations in the blood.

An "insulin secretagogue" is a compound which promotes increased secretion of insulin by the pancreatic beta cells. In general, an insulin secretor's action is initiated by binding to and closing a specific sulfonylurea receptor (an ATP-sensitive K^+ channel) on pancreatic β-cells which decreases K^+ influx, leading to depolarization of the membrane and activation of a voltage-dependent Ca^{2+} channel. Increased Ca^{2+} flux into the β-cell activates a cytoskeletal system that causes translocation of insulin to the cell surface and its extrusion by exocytosis. Insulin secretagogues include sulfonylureas, meglinitides, and amylin modulators.

A sulfonylurea useful for the methods and combinations of this invention may be a glyburide (DIABETA™), glipizide (GLUCOTROL™, GIBENESE™, MONODIAB™), glipizide (XL) (GLUCOTROL XL™), glimepiride (AMARYL™), glibenclamide (Daonil, Euglucon), gliclazide (Diamicron), gliquidone (Glurenorm), glibormuride, glisoxepide, glisentide, glisolamide, glyclopyamide, glycylamide, chlorpropamide (DIABINESE™), carbutamide, acetohexamide, tolbutamide, or tolazamide, or a pharmaceutically acceptable salt form of these agents. A combination of glyburide and metformin hydrochloride can also be commercially obtained under the GLUCOVANCE™ tradename (Bristol Meyers Squibb). Further suitable insulin secretagogues include repaglinide. Each of these agents may be produced by methods known in the art. These agents may also be administered at the pharmaceutically or therapeutically effective dosages or amounts known in the art for these compounds, such as those described in the Physician's Desk Reference 2001, 55 Edition, Copyright 2001, published by Medical Economics Company, Inc.

Meglinitides useful for the methods and combinations of this invention include repaglinide (Prandin®) and nateglinide (Starlix®).

An "amylin compound" refers to amylin and modulators thereof, in particular amylin agonists. The term "amylin" includes compounds such as those defined in U.S. Pat. No. 5,234,906 and U.S. Pat. No. 5,367,052. For example, it includes the human peptide hormone referred to as amylin and secreted from the beta cells of the pancreas, and species variations of it. Amylin is a 37 amino acid protein hormone that may include the sequence KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY (SEQ. ID. NOs. 44 and 45). The hormone is secreted along with insulin from the beta cells of the pancreas in response to a meal. [See for example, Rink et al., Trends in Pharmaceutical Sciences, 14:113-118 (1993); Gaeta and Rink, Med. Chem. Res., 3:483-490 (1994); and, Pittner et al., J. Cell. Biochem., 55S:19-28 (1994) for a review of the structure and biology of amylin.]

An "amylin agonist" refers to a compound that binds to or otherwise directly or indirectly interacts with an amylin receptor or other receptor or receptors with which amylin itself may interact to elicit a biological response, a compound mimics the function, activity, or effects of amylin, and/or peptide analogues of amylin useful as agonists of amylin. An amylin agonist may be a peptide or a non-peptide compound, and includes amylin agonist analogs. Amylin agonists useful in this invention include amylin agonist analogs disclosed and claimed in U.S. Pat. No. 5,686,411, U.S. Pat. No. 5,175,145, U.S. Pat. No. 6136784, and US Pat. No. 6,410,511, including without limitation ^{25,28,29}Pro-h-amylin (pramlintide, Symilin) [Amylin Pharmaceuticals (San Diego,

Calif., USA) and Johnson and Johnson (New Brunswick, N.J. USA.)], $^{18}Arg^{25,28,29}$Pro-h-amylin, and $^{18}Arg^{25,28}$Pro-h-amylin. Other amylin agonists include calcitonins and peptides or their equivalent having similar amino acid sequences to known calcitonins and having one or more of the known biological activities, in particular, the ability to increase circulating glucose concentration in humans. In an aspect the amylin agonist is Symlin. Amylin compounds may be produced by methods known in the art and they may be administered at therapeutically effective doses known in the art for the compounds.

An "antiobesity agent" or "appetite regulating agent" refers to any substance that may be used in the treatment or prevention of obesity or to modulate appetite in a subject. The agents may be produced by methods known in the art and they may be administered at therapeutically effective doses known in the art for the compounds. Examples of such agents include without limitation anorectic agents such as bromocryptine, dexfenfluramine and the like, monoamine reuptake inhibitors such as sibutramine and the like, sympathomimetics such as phendimetrazine and the like, fatty acid uptake inhibitors such as orlistat or the like, thyromimetics such as triiodothyronine or the like, CART (cocaine amphetamine regulated transcript) agonists, catecholaminergic agents (e.g. diethylpropion, phentermine, phenylpropanolamine, mazindol), NPY (neuropeptide Y) antagonists, MC 4 (meianocortin 4) agonists, MC 3 (melanocortin 3) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin releasing factor binding protein) antagonists, urocortin agonists, a melanin concentrating hormone antagonists, B_3 adrenergic receptor agonists, MSH (melanocyte-stimulating hormone) agonists or mimetics, INCH (melanocyte- concentrating hormone) antagonists, thyromimetic agents, dehydroepiandrosterone or an analog thereof, glucocorticoid receptor agonist or antagonist, ciliary neurotrophic factors, human agouti-related protein antagonists, CCK (cholecystokinin) agonists, monoamine re-uptake inhibitors, serotonin re-uptake inhibitors, serotonin and noradrenaline re-uptake inhibitors, mixed serotonin and noradrenergic compounds, 5HT (serotonin) agonists, dopamine agonists, bombesin agonists, galanin antagonists, growth hormone, growth factors such as prolactin or placental lactogen, growth hormone releasing compounds, TRH (thyreotropin releasing hormone) agonists, UCP 2 or 3 (uncoupling protein 2 or 3) modulators, leptin agonists, DA agonists (bromocriptin, doprexin), lipase/amylase inhibitors, RXR (retinoid X receptor) modulators, TR8 agonists, AGRP (Agouti related protein) inhibitors, opioid antagonists (such as naltrexone), PACAP (pituitary adenylyi cyclase activating peptide), cannabinoid receptor antagonists, and ciliary neurotrophic factor.

β_3-adrenergic receptor agonists include without limitation {4-[2-(2-[6 aminopyridin-3- yl]-2(R)-hydroxyethylamino)ethoxy]phenyl}acetic acid, {4-[2-(2-[6-aminopyridin-3- yl]-2(R)-hydroxyethylamino)ethoxy]phenyl}benzoic acid, {4-[2-(2-[6- aminopyridin-3- yl]-2(R)-hydroxyethylamino)ethoxy]phenyl}propionic acid, and {4-[2- (2-[6- aminopyridin-3-yl]-2(R)-hydroxyethylamino) ethoxy]phenoxy}acetic acid.

In an aspect of the invention the appetite regulating agent is an amphetamine-related appetite suppressant, in particular phentermine or phentermine hydrochloride (see U. S. Pat. No. 2,408,345). In another aspect of the invention the appetite regulating agent is the gut hormone peptide W (PW) (Batterham RL, Bloom SR, Ann N Y Acad Sci. (2003 Jun); 994:162-8), in particular the gut hormone fragment peptide YY3-36 peptide (W336).

In another aspect of the invention the antiobesity agent is leptin. In a further aspect of the invention the antiobesity agent is dexamphetamine or amphetamine.

In a further aspect of the invention the antiobesity agent is a serotonin agonist, in particular fenfluramine or dexfenfluramine or dexfenfluramine hydrochloride, in particular fenfluramine and dexfenfluramine (see U.S. Pat. No. 3,198,834).

In a further aspect of the invention the anti-obesity agent is a monamine reuptake inhibitor, in particular sibutramine or its hydrochloride salt (see U.S. Pat. No. 4,929,629), preferably in the form of Meridia™.

In a further aspect of the invention the antiobesity agent is a dopamine agonist, in particular bromocriptine (see U.S. Patent Nos. 3,752,814 and 3,752,888

In a further aspect of the invention the antiobesity agent is a lipase inhibitor, in particular dexfenfluramine hydrochloride or orlistat (see U.S. Pat. No. 4,598,089 and U.S. Pat. No. 6,004,996; orlistat is commercially available under the trade name Xenical™).

In a further aspect of the invention the antiobesity agent is mazindol or phentermine. Phentermine is commercially available under the trade name Ionamin™.

In a further aspect of the invention the antiobesity agent is phen-fen, which is a combination of fenfluramine or its hydrochloride and phentermin.

In a further aspect of the invention the antiobesity agent is phendimetrazine (Bontril™, X-Trozine™) or its tartrate salt, diethylpropion (Tenuate™) or its hydrochloride salt, fluoxetine, sertaline or its hydrochloride salt, ephedrine or its sulphate salt, bupropion, topiramate, benzphetamine or its hydrochloride salt, phenylpropanolamine or its hydrochloride salt, or ecopipam.

In an embodiment of the invention, an antiobesity agent comprises or is selected from the group consisting of phentermine, leptin, bromocriptine, dexamphetamine, amphetamine, fenfluramine, dexfenfluramine, sibutramine, orlistat, dexfenfluramine, mazindol, phentermine, phendimetrazine, diethylpropion, fluoxetine, bupropion, topiramate, diethylpropion, benzphetamine, phenylpropanolamine or ecopipam, ephedrine, pseudoephedrine and pharmacoutical salts thereof.

"Insulin" includes fast-, intermediate-, and long-acting insulins. In particular fast-acting insulins include regular insulins and prompt insulin zinc suspensions (semilente insulins); intermediate-acting insulins include isophane insulin suspensions (NPH insulins, isophane insulin) and the insulin zinc suspensions (lente insulins); and the long-acting insulins include protamine zinc insulin suspensions, and extended insulin zinc suspensions (ultralente insulins). The preparations may be available as either porcine or bovine insulins. The term includes recombinant human insulin available as regular and isophane insulins and as insulin zinc suspensions, as well as modified fast-acting insulin [Lys(B28), Pro(B29) human insulin analog, created by reversing the amino acids at positions 28 and 29 on the insulin B-chain].

Commercially available insulins include without limitation the fast-acting insulins available from Eli Lilly such as (a) Iletin® I (Regular); (b) Regular Iletin® II (Pork, 100 Units); (c) Regular Iletin® II (Concentrated, Pork, 500 Units); (d) Humalog® Injection (insulin lyspro, recombinant DNA origin); and (e) Humulin® R (regular insulin, recombinant DNA origin, 100 Units); the fast-acting insulins available from Novo Nordisk such as (a) Novolin® R (Regular, Human Insulin Injection (recombinant DNA origin) 100 Units); (b) Novolin® R PenFill 1.5 ml Cartridges (Regular, Human Insulin Injection (recombinant DNA origin) 100 Units);

(c) Novolin® R Prefilled.TM. (Regular, Human Insulin Injection (recombinant DNA origin) in a 1.5 ml Prefilled Syringe, 100 units/ml); (i) Regular Purified Pork Insulin (100 Units/ml); and (d) Velosulin® BR (Buffered Regular Human Insulin Injection, 100 Units/ml); the intermediate-acting insulins available from Eli Lilly such as (a) Humulin® 50/50 (50% human insulin isophane suspension and 50% human insulin injection (rDNA origin), 100 Units); (b) Humuline® 70/30 (70% human insulin isophane suspension and 30% human insulin injection (rDNA origin), 100 Units); (c) Humulin® L (lente; human insulin (rDNA origin) zinc suspension, 100 Units)); (d) Humulin® N (NPH; human insulin (rDNA origin) isophane suspension, 100 Units); (e) Lente® Iletin® I, (insulin zinc suspension, beef-pork); (f) NPH Iletin® I (isophane insulin suspension, beef-pork); (g) Lente Iletin® II (insulin zinc suspension, purified pork); and (h) NPH Iletin® II, (isophane insulin suspension, purified pork); the intermediate-acting insulins available from Novo Nordisk such as (a) Novolin® L (Lente, Human Insulin Zinc Suspension (recombinant DNA origin), 100 Units/ml); (b) Novolin® N (NPH, Human Insulin Isophane Suspension (recombinant DNA origin), 100 Units/ml); (c) Novolin® N PenFill® 1.5 ml Cartridges; (d) Novolin® N Prefilled™ (NPH, Human Insulin Isophane Suspension (recombinant DNA origin) in a 1.5 ml Prefilled Syringe, 100 Units/ml); (e) Novolin® 70/30 (70% NPH, Human Insulin Isophane Suspension and 30% Regular, Human Insulin Injection (recombinant DNA origin), 100 Units/ml); (f) Novolin® 70/30 PenFill® 1.5 ml Cartridges; (g) Novolin® 70/30 Prefilled™ (70% NPH, Human Insulin Isophane Suspension and 30% Regular, Human Insulin Injection (recombinant DNA origin) in a 1.5 ml Prefilled Syringe, 100 Units/ml); (h) Lente Purified Pork Insulin (Zinc Suspension, USP 100 Units/ml); and (i) NPH Purified Pork Isophane Insulin Suspension (100 Units/ml); and long acting insulins such as Eli Lilly's Humulin® U (Ultralente® human insulin (recombinant DNA origin) extended zinc suspension).

The structure of agents identified by generic or tradenames herein may be taken from the standard compendium "The Merck Index" or from databases such as PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi), and patent databases (http://www.uspto.gov/ patft/index.html; http://patents1.ic.gc.ca/intro-e.html; http://register.epoline.org/espacenet/ ep/en/srch-reg.htm). A person skilled in the art using these references is fully enabled to identify, manufacture and test the pharmaceutical indications and properties in standard test models, both *in vitro* and *in vivo*. The agents may also be administered at the pharmaceutically or therapeutically effective dosages or amounts known in the art for these compounds, such as those described in the Physician's Desk Reference 2001, 55 Edition, Copyright 2001, published by Medical Economics Company, Inc.

"Condition(s) and/or disease(s)" refers to one or more pathological symptoms or syndromes for which a gastrin agonist, a growth/hormone regulatory factor, and optionally a gastrin compound provide a beneficial or therapeutic effect. The condition and/or disease may require reduction of blood glucose levels, inhibition of gastric acid secretion, inhibition of apoptosis of β-cells, stimulation of proliferation or differentiation of β-cells, and reduction of body weight or insulin dependence. Examples of conditions and/or diseases include but are not limited to dyslipidemia, hyperglycemia, severe hypoglycemic episodes, stroke, left ventricular hypertrophy, arrhythmia, bacteraemia, septicaemia, irritable bowel syndrome, functional dyspepsia, diabetes, catabolic changes after surgery, stress induced hyperglycemia, respiratory distress syndrome, gastric ulcers, myocardial infarction, impaired glucose tolerance, hypertension, chronic heart failure, fluid retentive states, metabolic syndrome and related diseases, disorders, or conditions, obesity, diabetic complications as well as symptoms of

other diseases in which tissue is damaged due to elevated glucose levels, including Alzheimer's Disease, Parkinson's Disease, and other age-related, tissue-degenerative diseases, as well as the artherogenic effects of elevated leptin, for example in patients with impaired glucose tolerance and obese non-diabetic patients.

The term, "diabetes" as used herein means any manifested symptoms of diabetes in any mammal including experimental animal models, and including human forms such as Type I and Type II diabetes, early stage diabetes, and a pre-diabetic condition characterized by mildly decreased insulin or mildly elevated blood glucose levels. A "pre-diabetic condition" describes a subject demonstrating a symptom in terms of insulin or glucose level, and/or demonstrating a susceptibilty to diabetes or a related condition due to family history, genetic predisposition, or obesity in the case of Type II diabetes, and includes a subject who has previously had diabetes or a related disease, disorder, or condition and is subject to risk of recurrence.

Diseases, disorders, and conditions related to diabetes, in particular Type II diabetes, include without limitation, diabetic nephropathy, diabetic retinopathy and diabetic neuropathy, macular degeneration, coronary heart disease, myocardial infarction, diabetic cardiomyopathy, myocardial cell death, coronary artery diseases, peripheral arterial disease, stroke, limb ischemia, vascular restenosis, foot ulcerations, endothelial dysfunction and/or atherosclerosis.

In aspects of the invention, a condition and/or disease may be selected from the group consisting of (a) Type I or Type II diabetes mellitus and related diseases, disorders or conditions (including but not limited to diabetic nephropathy, diabetic retinopathy and diabetic neuropathy); (b) insulin resistance and syndrome X, obesity and related diseases, disorders or conditions (including but not limited to not limited to Insulin Resistance, Type 2 Diabetes Mellitus, Reproductive Disorders, Cardiovascular Disease, Pulmonary Disease, Gallstones and Fasting-induced cholecystitis, Cancers and Cutaneous Disease), Cushing's Syndrome, Hypothyroidism, Insulinoma, Craniopharyngioma and Other Disorders Involving the Hypothalamus; (c) congestive heart failure, left ventricular hypertrophy, survival post myocardial infarction (MI), coronary artery diseases, atherosclerosis, angina pectoris, thrombosis, (d) hypertension including hypertension in the elderly, familial dyslipidemichypertension and isolated systolic hypertension (ISH); increased collagen formation, fibrosis, and remodeling following hypertension (antiproliferative effect of the combination); impaired vascular compliance, stroke; all these diseases or conditions associated with or without hypertension, (e) renal failure, in particular chronic renal failure, glomerulosclerosis, nephropathy; (f) hypothyroidism; (g) endothelial dysfunction with or without hypertension, (h) hyperlipidemia, hyperlipoproteinemia, hypertryglyceridemia, and hypercholesterolemia, (i) macular degeneration, cataract, glaucoma, o) skin and connective tissue disorders, and (k) restenosis after percutaneous transluminal angioplasty, and restenosis after coronary artery bypass surgery; and peripheral vascular disease.

"Insulinotropic activity" refers to an ability of a substance to stimulate insulin secretion in response to elevated glucose levels to produce or increase glucose uptake by cells and decreased serum glucose or blood glucose levels. Methods known in the art can be employed to assay for insulinotropic activity. For example, *in vitro* and *in vivo* methods may be used that measure GLP-1 receptor binding activity or gastrin receptor binding activity, receptor activation (see the methods described in EP 619,322 to Gelfand et al and US Patent No. 5,120,712), and/or insulin or C-peptide levels. Compounds, compositions or conjugates described herein have insulinotropic activity if islet cells secrete insulin in the presence of the compounds, compositions, or conjugates

above background levels or levels in the absence of the compounds, compositions, or conjugates. A compound may be administered to an animal and the insulin concentration can be monitored over time.

"Islet neogenesis" means formation of new beta cells by differentiation, which may or may not have the characteristics of stem cells which have the ability to reproduce in an unlimited manner.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The invention is related to compositions, conjugates, and methods that utilize at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound. In particular, the invention relates to compositions, conjugates, and methods for the prevention, intervention and/or treatment of a condition and/or disease discussed herein comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound to provide one or more beneficial effect. In aspects of the invention, the compositions, conjugates and methods of the invention provide enhanced beneficial effects, in particular sustained beneficial effects relative to a gastrin agonist, growth/hormone regulatory factor, and optionally gastrin compound alone. The beneficial effects may be additive, complementary or synergistic effects.

In aspects of the invention, where the condition and/or disease is diabetes, beneficial effects, in particular sustained beneficial effects of a composition, combination treatment, or conjugate of the invention may manifest as one or more of the following:

a) An increase in pancreatic insulin levels relative to the levels measured in the absence of the active compounds or for each compound alone after administration to a subject with symptoms of diabetes. Preferably the compounds together induce at least about a 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, or 50% increase in pancreatic insulin levels in a subject.

b) A reduction of an absence of symptoms of islet inflammation after administration to a subject with symptoms of diabetes.

c) A decrease in blood glucose levels relative to the levels measured in the absence of the compounds or for each compound alone in subjects with symptoms of diabetes. Preferably, the compounds induce at least about a 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% decrease in blood glucose levels. Most preferably, the compounds yield blood glucose levels about or close to the levels common in a normal subject.

d) An increase in C-peptide levels relative to the levels measured in the absence of the compounds or for each compound alone in subjects with symptoms of diabetes. Preferably, the compounds together induce at least about a 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, or 50% increase in C-peptide levels.

e) Maintenance of blood glucose levels at about normal for a prolonged period of time, in particular for a period of at least 2, 4, 6, 8, or 10 weeks, 2 to 4 weeks, 2 to 6 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment.

f) Maintenance of hemoglobin A1c or glycated hemoglobin at about normal levels for a prolonged period of time, in particular maintaining a % hemoglobin A1c at between 6-8%, more particularly at about 7%.

g) A reduction in destruction of beta-cells. Preferably the compounds induce at least about a 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in destruction of beta-cells.

h) An increase in beta-cell function. Preferably the compounds induce at least about a 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% increase in beta-cell function.

i) A decrease in insulin delivery or usage compared with the absence of the compounds or for each compound alone in diabetic subjects. Preferably, the compounds provide at least about a 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 93%,94%, 95%, 96%, 97%, 98%, 99%, 100%, 30-100%, 30-80%, or 35-75%, reduction in insulin delivery or usage.

j) A decrease in requirement for insulin injection/intake by at least 5-99%, 5-95%, 10-98%, 10-95%, 10-90%, 10-80%, 10-70%, 10-60%, 10-50%, 10-40%, 10-30%, 10-20%, 20-100%, 20-75%, 30-100% 30-90%, 30-80%, 30-75%, 35-90%, 35-80%, or 35-75%.

k) A reduction, prevention, or slowing of the rate of disease progression in a subject with diabetes.

l) A reduction or prevention of the development of severe hyperglycemia and ketoacidosis with symptoms of diabetes.

m) An increase in survival in a subject with symptoms of diabetes.

In embodiments of the invention, beneficial effects or sustained beneficial effects comprise or consist essentially of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or thirteen of a) through m). In particular embodiments, beneficial effects or sustained beneficial effects comprise or consist essentially of a), b), and c); a), b), c), and d); a), b), c), d), and e); a), b), c), d), e), and f); a), b), c), d), e), f), and g); a), b), c), d), e), f), g), and h); a), b), c), d), e), f), g), h), and i); a), b), c), d), e), f), g), h), i) and j); a), d), and e); a), d), e), and h); a), d), e), h), and i); a), d), e), h), i), and j); a), b), c), d), e), h), i), and j); a), b), c), d), e), h), i), j), and k); b), c), d), and e); b), c), d), e), h), i), and j); b), h), i) and j); a) through e); a) through f); a) through g); a) through h); a) through i); a) through j); a) through k); a) through l); and a) through m).

One or more of these beneficial effects or sustained beneficial effects can be demonstrated in a diabetic subject or disease model, for example a non-obese (NOD) mouse with symptoms of diabetes, using standard methods known to the skilled artisan. For example, commercially available methods and kits may be used to assay pancreatic insulin levels, glucose levels, C-peptide levels and hemoglobin A1c.

A gastrin agonist may be selected for particular applications in the present invention based on its ability to increase plasma gastrin, about 1 to 1000 fold, 10 to 1000 fold, 5 to 500 fold, 10 to 500 fold, 10 to 200 fold, 10 to 150 fold, 10 to 100 fold, 10 to 75 fold, 10 to 50 fold, 10 to 40 fold, 10 to 30 fold or 10 to 20 fold. In aspects of the invention a gastrin agonist is selected that has a bioavailability of greater than 40%, 50%, 60%, 70%, 80% or 90% and/or has a plasma elimination half-life of greater than 1 hour.

In certain aspects of the invention the gastrin agonist is a proton pump inhibitor. In embodiments of the invention the proton pump inhibitor is leminoprazole, nepaprazole, tenatoprazole, lansoprazole, rabeprazole, pantoprazole, pariprazole, (−)pantoprazole, soraprazan, ilaprazole, AZD-0865, hydroxyomeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereo.

In certain aspects of the invention, the gastrin agonist is one or more of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereo.

In particular aspects of the invention, the gastrin agonist is selected from the group consisting of one or more of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereof.

A growth/hormone regulatory factor may be selected for particular applications in the present invention based on one or more of the following characteristics: ability of the growth/hormone regulatory factor to bind to its receptor with an affinity constant K_d less than about 1 μM; insulinotropic activity; stimulation of beta cell proliferation/differentiation; resistance to DPP IV cleavage; and, an *in vivo* half-life of at least about 15 minutes to 24 hours, preferably 2 to 10 hours or 2 to 8 hours in humans.

In aspects of the invention the growth/hormone regulatory factor is an EGF receptor ligand. In particular aspects, an EGF receptor ligand is represented as A-B wherein A comprises amino acids 1-53 of SEQ ID NO: 36 and wherein at least one amino acid is replaced at positions 48-53 of the carboxy terminus, the amino acid sequence being more stable to proteolysis than that of SEQ ID NO: 36. In another particular aspect, an EGF receptor ligand is utilized wherein A comprises amino acids 1-50 of SEQ ID NO: 36 and B is aspargine (i.e., EGF 1-51 $glu^{51}asn$ or Asn^{51}-hEGF51, SEQ ID NO. 37).

In aspects of the invention the growth/hormone regulatory factor is a GLP-1 agonist, in particular a naturally truncated GLP-1 polypeptide (GLP-1(7-36) or ((GLP-1(7-37)), or an analogue or derivative thereof. The sequences of these naturally occurring truncated GLP-1 agonists are represented in SEQ ID NOs. 18, 19, and 20.

In certain aspects of the invention, a GLP-1 agonist may have the amino acid sequence of SEQ ID NOs. 17, 18, or 19 modified so that amino acid residues at positions 1-20, preferably 1-15, more preferably 1-10, most preferably 1-5 differ from the sequences of SEQ ID NOs. 17, 18 or 19.

In an embodiment of the invention, the GLP-1 agonist is an analogue of GLP-1(7-37) or GLP-1(7-36) which has less than 10 amino acid residues that are different from those in GLP-1(7-37) or GLP-1(7-36), less than 5 amino acid residues that are different from those in GLP-1(7-37) or GLP-1(7-36), less than 3 amino acid residues that are different from those in GLP-1 (7-37) or GLP-1(7-36), preferably only one amino acid residue that is different from the sequence of GLP-1(7-37) or GLP-1(7-36).

GLP-1 agonists that may have specific utility in the present invention include polypeptides where one or more amino acids have been added to the N-terminus and/or C-terminus of GLP-1(7-37) or GLP-1(7-36). Preferably, about one to six amino acids may be added to the N-terminus and/or from about one to eight amino

acids may be added to the C-terminus. In certain applications GLP-1 agonists are selected that have up to 39 amino acids. Amino acids at positions 1-6 of an extended GLP-1 agonist may be selected so that they are the same or are conservative substitutions of the amino acid at the corresponding positions of the parent GLP-1(7-37) or GLP-1(7-36). Amino acids at positions 38-45 of an extended GLP-1 agonist may be selected so that they are the same or conservative substitutions of the amino acids at the corresponding positions of exendin-3 or exendin-4 (SEQ ID NO. 22 and 23, respectively).

In aspects of the invention a GLP-1 agonist is utilized comprising a position 8 analogue wherein the backbone for such analogs or fragments thereof contains an amino acid other than alanine. The amino acid at position 8 may be selected from glycine, valine, leucine, isoleucine, serine, threonine, or methionine.

In an embodiment a GLP-1 agonist is an insulinotropic analogue of GLP-1(1-37), for example, Met^{8}-GLP-1(7-37), wherein the alanine in position 8 has been replaced by methionine and the amino acid residues in position 1 to 6 have been deleted, and Arg^{34}-GLP-1(7-37) wherein the valine in position 34 has been replaced with arginine and the amino acid residues in position 1 to 6 have been deleted.

In another embodiment, GLP-1 agonists are selected that have the sequence GLP-1(7-37)OH and GLP-1(7-36) amide, and the corresponding position 8 analogs wherein the backbone for such analogs contains an amino acid other than alanine. The amino acid at position 8 may be selected from glycine, valine, leucine, isoleucine, serine, threonine, or methionine, preferably valine or glycine. The analogs may additionally contain (a) an amino acid at position 22 selected from glutamic acid, lysine, aspartic acid, arginine, and preferably glutamic acid or lysine; (b) an amino acid at position 30 selected from glutamic acid, aspartic acid, serine, or histidine; (c) an amino acid at position 37 selected from lysine, arginine, threonine, glutamic acid, aspartic acid, serine, tryptophan, tyrosine, phenylalanine, or histidine; and/or (d) amino acid at position 27 selected from alanine, lysine, arginine, tryptophan, tyrosine, phenylalanine, or histidine.

A group of GLP-1 analogs and derivatives for use in the present invention comprises the GLP-1 agonists described in U.S. Pat. No. 5,545,618 and US Patent Application Serial No. 20040018975. The analogs include active GLP-1 peptides, 7-34, 7-35, 7-36 and 7-37 having amino acid substitutions at positions 7-10 and/or are truncations at the C-terminus and/or contain various other amino acid substitutions in the basic peptide. Preferred analogs include those with D-amino acid substitutions in the 7 and 8 positions and/or N-alkylated or N-acylated amino acids in the 7 position since they are particularly resistant to degradation *in vivo*.

In aspects of the invention, a GLP-1 agonist comprises a peptide comprising or selected from the group consisting of GLP-1 (1-38); GLP-1 (1-39), GLP-1 (1-40), GLP-1 (1-41), GLP-1 (7-38), GLP-1 (7-39), GLP-1 (7-40), and GLP-1 (7-41).

In another aspect of the invention at least one amino acid of a GLP-1 agonist has at least one substituent attached directly or indirectly (e.g. via a spacer such as γ-Glu or β-Ala). A substituent is generally selected to make the profile of action of the parent GLP-1 agonist more protracted, make the GLP-1 agonists more metabolically and physically stable, and/or increase solubility of the GLP-1 agonist. An example of a particular substituent is an amide, a carbohydrate, and a lipophilic substituent. A lipophilic substituent includes but is not limited to an alkyl group, a group which has an ω-carboxylic acid group, an acyl group of a straight-chain or branched fatty acid or alkane such as tetradecanoyl, hexadecanoyl. Particular compositions, conjugates and

treatments of the invention use GLP-1 agonists with lipophilic substitutents such as those described in W0 99/43341 (Novo Nordisk) and US 2003/0119734A1 (Novo Nordisk).

In particular aspects of the invention a GLP-1 agonist is a GLP-1(7-36)-amide or Tyr^{31}-exendin-4(1-31)-amide.

Certain aspects of the invention provide a GLP-1 agonist that is a derivative of GLP-1 (7-36) or GLP-1 (7-37) comprising a lipophilic substitutent. In an embodiment, the GLP-1 agonist is $Arg^{34}Lys^{26}(N^{\varepsilon}(\gamma\text{-}Glu(N^{\alpha}\text{-}hexadecanoyl)))$-GLP-1(7-37).

In a particular embodiment a GLP-1 agonist comprises or is selected from the group consisting of Val^{8}-GLP-1(7-37)OH, Gly^{8}-GLP-1(7-37)OH, Glu^{22}-GLP-1(7-37)OH, Lys^{22}-GLP-1(7-37)OH, Val^{8}-Glu^{22}-GLP-1(7-37)OH, Val^{8}- Lys^{22}-GLP-1(7-37)OH, Gly^{8}-Glu^{22}-GLP-1(7-37)OH, Gly^{8}-Lys^{22}-GLP-1(7-37)OH, Glu^{22}-GLP-1(7-36)NH_2, Lys^{22}-GLP-1(7-36)NH_2, Val^{8}-Glu^{22}-GLP-1(7-36)NH_2, Val^{8}-Lys^{22}-GLP-1(7-36)NH_2, Gly^{8}-Glu^{22}-GLP-1(7-36)NH_2, Gly^{8}-Lys^{22}-GLP-1(7-36)NH_2, Val^{8}-His^{37}-GLP-1(7-37)OH, Gly^{8}-His^{37}-GLP-1(7-37)OH, Arg^{34}-GLP-1(7-36)NH_2, and Arg^{34}-GLP-1(7-37)OH.

In another particular embodiment, the GLP-1 agonist comprises or is selected from the group consisting of Gly^{8}-GLP-1(7-37), Val^{8}GLP-1(7-37), $Val^{8}Asp^{22}$GLP-1(7-37), $Val^{8}Glu^{22}$GLP-1(7-37), $Val^{8}Lys^{22}$GLP-1(7-37), and $Val^{8}His^{22}$ GLP-1(7-37), and analogs and derivatives thereof.

In a further particular embodiment, the GLP-1 agonist comprises or is selected from the group consisting of Gly^{8}-GLP-1(7-36) amide, Val^{8}GLP-1(7-36) amide, $Val^{8}Asp^{22}$GLP-1(7-36) amide, $Val^{8}Glu^{22}$GLP-1(7-36) amide, $Val^{8}Lys^{22}$GLP-1(7-36) amide, and $Val^{8}His^{22}$ GLP-1(7-36) amide, and analogs and derivatives thereof.

In still further embodiments of the invention, a GLP-1 agonist is exendin (e.g. exendin 3 and exendin 4) or an analog, derivative, or fragment thereof. Examples of exendins that may be utilized in the present invention include without limitation those disclosed in WO 9746584, US Patent No. 5,424,286 and WO 01/04156. US Patent No. 5,424,286 describes use of an exendin polypeptide for stimulating insulin release. The exendin polypeptides described in the US patent include HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGX wherein X=P or Y, and HX_1X^2GTFITSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS; wherein X^1X^2=SD (exendin-3) or GE (exendin-4). WO 9746584 describes truncated exendin peptides that increase secretion and biosynthesis of insulin but reduce glucagons. WO 01/04156 describes analogs and derivatives of exendin-4 and their preparation. In particular embodiments, the GLP-1 agonist is exendin-4, in particular exenatide, more particularly Byetta™.

In another embodiment, a GLP-1 agonist is an insulinotropic analogue of exendin-4(1-39), in particular $Ser^{2}Asp^{3}$-exendin-4(1-39) wherein the amino acid residues in position 2 and 3 have been replaced with serine and aspartic acid, respectively (this particular analogue is also being known in the art as exendin-3, SEQ ID NO. 22).

In certain aspects of the invention the GLP-1 agonist is a stable GLP-1 agonist in particular a stable GLP-1 analogue or derivative, or a stable exendin-4 or exendin-3 analogue or derivative.

In aspects of the invention, a composition, conjugate or method utilizes two or more growth/hormone regulatory factors. In a particular aspect, an EGF receptor ligand and a GLP-1 agonist are utilized.

A gastrin compound may be selected for particular embodiments in the present invention and to provide a specific beneficial effect(s) based on characteristics including its insulinotrophic activity, the ability to augment

the activity of a growth/hormone regulatory factor (in particular to enhance the insulinotropic effects of a growth/hormone regulatory factor), and/or increase the physical or chemical stability of a growth/hormone regulatory factor. A gastrin compound can also be selected based on its ability to stimulate proliferation/differentiation of beta cells, and its *in vivo* half-life.

A gastrin compound used in aspects of the methods, compositions, and conjugates of the invention is gastrin 17 and analogs and derivatives thereof, associated with a polymer. In particular aspects, the gastrin compound is synthetic human gastrin I having 17 amino acid residues with a Leu residue at amino acid position 15 [SEQ ID NO. 4].

In another aspect of the invention, a gastrin compound used in the methods, compositions and conjugates of the invention is gastrin 34 and analogs and derivatives thereof. In a particular aspect, the gastrin compound is a synthetic human gastrin 34 with methionine or leucine at position 32 [SEQ ID NO. 1 or 2].

In a further aspect of the invention, a gastrin compound used in the methods, compositions and conjugates of the invention is gastrin 34 or gastrin 17 or portions thereof, directly or indirectly interacting or associated with a serum protein, in particular albumin or an immunoglobulin, more particularly human serum albumin.

In particular aspects of the invention, a gastrin compound comprises synthetic human gastrin 34 having 2-34 amino acid residues of SEQ ID NO. 1 or 2, and optionally an N-terminal cysteine and/or a carrier; synthetic human gastrin having 1-17 amino acid residues with a Leu residue at amino acid position 15 [SEQ ID NO. 4] and optionally an N-terminal cysteine residue; and a synthetic human gastrin having amino acid residues 2 to 17 or 5-17 of SEQ ID NO. 3 or 4, optionally with an N-terminal cysteine residue and/or a carrier (e.g. PEG or human serum albumin) linked via a spacer [e.g. Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala i.e. $(GA)_5$] [SEQ ID NO. 2], in particular, a synthetic human gastrin having amino acid residues 2 to 17 or 5-17 of SEQ ID NO. 3 or 4, with a human serum albumin (HSA) polymer linked via a Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala [i.e., $(GA)_5$] spacer, and optionally an N-terminal cysteine residue.

Pharmaceutical compositions of the invention can be selected that provide beneficial effects, in particular statistically significant beneficial effects or sustained beneficial effects, compared with one or two of a gastrin agonist, growth/hormone regulatory factor and gastrin compound. Beneficial effects in respect to a diabetic condition may be evidenced by one or more of the beneficial effects described herein, in particular one, two, three, four, five, six, seven, eight, nine or ten of the beneficial effects described above in a) through l).

In an aspect, a pharmaceutical composition is provided comprising a gastrin agonist and an EGF receptor ligand. In a particular pharmaceutical composition, the EGF receptor ligand is represented by A-B wherein A comprises amino acids 1-50 of SEQ ID NO: 36 and B is aspargine (EGF 1-51 $glu^{51}asn$ or Asn^{51}-hEGF51). In a particular aspect, the gastrin agonist is selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereof.

In an aspect, the pharmaceutical composition comprises a gastrin agonist and a GLP-1 agonist. In a particular aspect, the GLP-1 agonist is selected from the group consisting of Gly^8-GLP-1(7-37), Val^8GLP-1(7-37), Val^8Asp^{22}GLP-1(7-37), Val^8Glu^{22}GLP-1(7-37), Val^8Lys^{22}GLP-1(7-37), Val^8His^{22} GLP-1(7-37),

$Arg^{34}Lys^{26}(N^{\varepsilon}(\gamma\text{-}Glu(N^{\alpha}\text{-}hexadecanoyl)))$-GLP-1(7-37), Gly^{8}-GLP-1(7-36) amide, Val^{8}GLP-1(7-36) amide, $Val^{8}Asp^{22}$GLP-1(7-36) amide, $Val^{8}Glu^{22}$GLP-1(7-36) amide, $Val^{8}Lys^{22}$GLP-1(7-36) amide, and $Val^{8}His^{22}$ GLP-1(7-36) amide. In other aspects, the GLP-1 agonist is exendin-4, in particular exenatide, more particularly Byetta™.

In particular aspects, a pharmaceutical composition with statistically significant beneficial effects or sustained beneficial effects is provided comprising a gastrin agonist selected from the group consisting of esomeprazole, tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereof, and a GLP-1 agonist and/or an EGF receptor ligand.

In particular aspects, a pharmaceutical composition with statistically significant beneficial effects or sustained beneficial effects is provided comprising a gastrin agonist selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereof, $Arg^{34}Lys^{26}(N^{\varepsilon}(\gamma\text{-}Glu(N^{\alpha}\text{-}hexadecanoyl)$-GLP-1(7-37) and a gastrin-17(leu) [SEQ ID NO.14].

In other aspects, a pharmaceutical composition with statistically significant beneficial effects or sustained beneficial effects is provided comprising a gastrin agonist selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereof, $Aib^{8,35}$ GLP-1(7-36) amide, Ser^{38},$Lys^{39,40,41,42,43,44}$-Exendin-4(1-39)amide or Byetta™, and optionally gastrin-17(leu) [SEQ ID NO.14].

In certain aspects of the invention, pharmaceutically acceptable salts of a gastrin agonist, growth/hormone regulatory factor and/or a gastrin compound are utilized.

The invention in particular aspects provides a pharmaceutical composition which has been adapted for administration to a subject to provide sustained beneficial effects to treat a condition and/or disease, preferably diabetes. In an embodiment for the prevention and/or treatment of diabetes, the composition is in a form such that administration to a subject results in blood glucose levels that are about normal or lower and that persist in the subject for a prolonged period of time after cessation of treatment.

This invention provides a conjugate comprising one or more of a gastrin agonist linked to or interacting with a growth/hormone regulatory factor, and optionally a gastrin compound wherein the interaction is for example, via an amino or a carboxyl group. The invention also relates to isolated covalent conjugates of the invention, and compositions comprising covalent conjugates of the invention. A growth/hormone regulatory factor may be conjugated to a species via an ester bond between an OH and a COOH of a gastrin agonist and optionally a gastrin compound. Conjugates may be conjugated with an intermediate spacer or linker. A suitable spacer or linker may be a mono- or disaccharide, an amino acid, a sulfate, a succinate, an acetate, or an oligomeric polymeric spacer or linker comprising one or more of such moieties.

The invention also provides methods of preparing conjugates with improved pharmacokinetic properties, biological activity, and beneficial effects. The methods comprise incubating a gastrin agonist with a

growth/hormone regulatory factor, and optionally a gastrin compound under conditions that allow formation of a covalent linkage between the compounds. The invention therefore contemplates a process for preparing a covalent conjugate comprising a gastrin agonist covalently bonded or linked to a growth/hormone regulatory factor and optionally a gastrin compound, the process comprising: incubating a gastrin agonist with a growth/hormone regulatory factor and optionally a gastrin compound under conditions and at a pH and for a time sufficient for formation of a covalent bond or linkage between the gastrin agonist and growth/hormone regulatory factor; and isolating the covalent conjugate. The above process for preparing a conjugate may provide a conjugate with a substantial amount of a gastrin agonist covalently linked to a growth/hormone regulatory factor.

N-terminal or C-terminal fusion proteins or chimeric proteins, comprising a growth/hormone regulatory factor conjugated with a gastrin agonist and optionally a gastrin compound, optionally with spacers or linkers, may be prepared by fusing, through recombinant techniques, the N-terminal or C-terminal sequence of a growth/hormone regulatory factor and the sequence of a gastrin agonist, and optionally a sequence of a gastrin compound.

The invention relates to a conjugate prepared by a process described herein. The invention also relates to pharmaceutical formulation or composition comprising conjugates of the invention and a pharmaceutically acceptable carrier, excipient, or vehicle. The invention further relates to a pharmaceutical formulation or composition of substantially pure covalent conjugates comprising a gastrin agonist covalently linked to a growth/hormone regulatory factor and optionally a gastrin compound which provides beneficial effects preferably sustained beneficial effects compared to the compounds alone. In an embodiment, a pharmaceutical formulation is provided consisting essentially of covalent conjugates comprising a gastrin agonist covalently linked without an intermediate spacer or linker to a growth/hormone regulatory factor. In another embodiment, a pharmaceutical formulation is provided consisting essentially of covalent conjugates comprising a gastrin agonist covalently linked with an intermediate spacer or linker to a growth/hormone regulatory factor and optionally a gastrin compound.

In aspects of the invention, a composition or conjugate comprising a gastrin agonist and a growth/hormone regulatory factor, and optionally a gastrin compound, have greater sustained insulinotropic activity following treatment compared with the activity of the compounds alone.

The invention provides methods for the prevention, treatment and/or intervention of a condition and/or disease in a subject comprising administering at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound or a pharmaceutical composition of the invention to provide a beneficial effect, in particular a sustained beneficial effect.

In methods of the invention a gastrin agonist is selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereof

In methods of the invention, the growth/hormone regulatory factor is a GLP-1 agonist. A GLP-1 agonist can be selected from the group consisting of Gly^{8}-GLP-1(7-37), Val^{8}GLP-1(7-37), $Val^{8}Asp^{22}$GLP-1(7-37), $Val^{8}Glu^{22}$GLP-1(7-37), $Val^{8}Lys^{22}$GLP-1(7-37), $Val^{8}His^{22}$ GLP-1(7-37), $Arg^{34}Lys^{26}(N^{\varepsilon}(\gamma\text{-Glu}(N^{\alpha}\text{-}$ hexadecanoyl))-GLP-1(7-37), Gly^{8}-GLP-1(7-36) amide, Val^{8}GLP-1(7-36) amide, $Val^{8}Asp^{22}$GLP-1(7-36) amide,

Val^8Glu^{22}GLP-1(7-36) amide, Val^8Lys^{22}GLP-1(7-36) amide, and Val^8His^{22} GLP-1(7-36) amide. In particular embodiments, the GLP-1 agonist is exendin-4, in particular exenatide, more particularly Byetta™.

In other methods of the invention the growth/hormone regulatory factor is an EGF receptor ligand represented by A-B wherein A comprises amino acids 1-50 of SEQ ID NO: 36 and B is aspargine (i.e., EGF 1-51 $glu^{51}asn$ or Asn^{51}-hEGF51).

In certain methods of the invention a gastrin compound is utilized comprising SEQ ID NO. 1, 2, 13 or 4, optionally associated with a serum protein.

In certain methods of the invention a gastrin agonist is selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereof and GLP-1(7-36) [SEQ ID NO. 20] or exenatide, more particularly Byetta™, are administered.

In certain other methods of the invention a gastrin agonist is selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, and leminoprazole, $Arg^{34}Lys^{26}(N^{\varepsilon}(\gamma\text{-Glu}(N^{\alpha}\text{-hexadecanoyl})$-GLP-1(7-37) and optionally gastrin-17(leu) [SEQ ID NO.4], are administered.

In certain other methods of the invention a gastrin agonist selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, and leminoprazole, $Aib^{8,35}$ GLP-1(7-36) amide, Ser^{38},$Lys^{39,40,41,42,43,44}$-Exendin-4(1-39)amide, or exenatide, more particularly Byetta™, and optionally gastrin-17(leu) [SEQ ID NO.4], and a gastrin agonist are administered.

In certain further methods of the invention providing beneficial effects, in particular statistically significant beneficial effects or sustained beneficial effects, a gastrin agonist is selected from the group consisting of tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, and leminoprazole, a GLP-1 agonist is selected from the group consisting of Gly^8-GLP-1(7-37), Val^8GLP-1(7-37), Val^8Asp^{22}GLP-1(7-37), Val^8Glu^{22}GLP-1(7-37), Val^8Lys^{22}GLP-1(7-37), Val^8His^{22} GLP-1(7-37), $Arg^{34}Lys^{26}(N^{\varepsilon}(\gamma\text{-Glu}(N^{\alpha}\text{-hexadecanoyl})))$-GLP-1(7-37), Gly^8-GLP-1(7-36) amide, Val^8GLP-1(7-36) amide, Val^8Asp^{22}GLP-1(7-36) amide, Val^8Glu^{22}GLP-1(7-36) amide, Val^8Lys^{22}GLP-1(7-36) amide, Val^8His^{22} GLP-1(7-36) amide, and exenatide, more particularly Byetta™, and optionally a gastrin compound comprises an amino acid sequence comprising, from the amino terminus, $Z\text{-}Y_m\text{-}X_n\text{-}AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6$, wherein AA_1 is Tyr or Phe, AA_2 is Gly, Ala, or Ser, AA_3 is Trp, Val, or Ile, AA_4 is Met or Leu, AA_5 is Asp or Glu, and AA_6 is Phe or Tyr; Z is a polymer and when the polymer is a protein Z is an amino acid sequence; Y_m is an optional spacer region comprising m amino acid residues of a small neutral amino acid including but not limited to serine and alanine, and X is any consecutive portion of residues 1-28 of SEQ ID NO: 1 or 2, or residues 1-17 of SEQ ID NO. 3 or 4, preferably $AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6$ is Tyr-Gly-Trp-Met-Asp-Phe or Tyr-Gly-Trp-Leu-Asp-Phe.

In aspects, the invention provides compositions and methods comprising a gastrin agonist, a growth/hormone regulatory factor and a gastrin compound. In particular aspects, a gastrin compound is selected

from the group consisting of gastrin 71 [SEQ ID NO. 5, residues 22 to 92], gastrin 52 [SEQ ID NO. 6], gastrin 34 (big gastrin) [SEQ ID NO. 1 or 2], gastrin 17 (little gastrin) [SEQ ID NO. 3 or 4], gastrin 14 [SEQ ID NO. 7], gastrin 8, gastrin 6 [SEQ ID NO. 8 or 9], pentagastrin, and tetragastrin, preferably 15Leu gastrin 17 [SEQ ID NO. 4]. In embodiments of this aspect of the invention the gastrin agonist is a proton pump inhibitor, in particular one or more of omeprazole, hydroxyomeprazole, esomeprazole, tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereto.

In an aspect, the invention provides a method for the prevention and/or intervention of a condition and/or disease discussed herein in a subject comprising administration of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound. The compounds may be directly administered to a subject or contacted with cells (e.g. stem cells or progenitor cells) and administered to a subject.

The invention also provides a combination treatment for preventing and/or treating a condition and/or disease discussed herein in a subject comprising administering to the subject a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound to provide beneficial effects. In an aspect the invention provides a combination treatment or intervention which provides sustained beneficial effects following treatment.

In particular, the invention provides a combination treatment for treating or preventing a condition and/or disease in a subject comprising administering to the subject a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound to produce beneficial effects, preferably sustained beneficial effects.

The invention also relates to a method of treatment comprising administering a therapeutically effective amount of at least one gastrin agonist in combination with the administration of at least one growth/hormone regulatory factor, and optionally at least one gastrin compound which upon administration to a subject with symptoms of diabetes produces beneficial effects, preferably sustained beneficial effects, manifested as reduced blood glucose levels and/or increased pancreatic insulin.

In an aspect of the invention therapeutically effective amounts of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound are combined prior to administration to a subject. In an embodiment, therapeutically effective amounts of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound are mixed at a physiologically acceptable pH.

In an embodiment, the invention provides a method for stimulating beta cell proliferation in a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound.

In another embodiment, the invention provides a method for increasing the number and/or size of beta cells in a subject comprising administering a therapeutically effective amount of a composition or conjugate of

the invention or administering in combination at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound.

In a further embodiment, the invention provides a method for preventing or treating Type I or Type II diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound.

In a still further embodiment, the invention provides a method for amelioriating progression of disease or obtaining a less severe stage of disease in a person suffering from Type I or Type II diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination, at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound.

The invention relates to a method of delaying the progression of impaired glucose tolerance or non-insulin requiring Type II diabetes to insulin requiring Type II diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound.

The invention also relates to a method of increasing the insulin synthesis capability of a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination, at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound.

The invention further relates to inducing islet neogenesis in a subject comprising contacting islet precursor cells with at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, composition, or conjugate of the invention in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

The invention contemplates a method of expanding a functional beta cell mass of pancreatic islet transplants in a diabetic patient, the method comprising administering to the patient a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention.

In an aspect, the invention provides methods for treating diabetes mellitus in a patient in need thereof by administering a composition comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound in an amount sufficient to effect differentiation of the patient's pancreatic islet precursor cells to mature insulin-secreting cells and/or to stimulate insulin synthesis in existing islet cells. The composition can be administered systemically or expressed *in situ* by host cells containing one or more nucleic acid construct in an expression vector wherein the nucleic acid construct comprises a coding sequence for a gastrin agonist and a growth/hormone regulatory factor and optionally a gastrin compound, together with transcriptional and translational regulatory regions functional in pancreatic islet precursor cells.

In an aspect, the invention provides a method for treating diabetes in a subject receiving insulin, and one or more glucose lowering agent comprising administering a therapeutically effective amount of a growth/hormone regulatory factor and a gastrin compound. In an embodiment of the invention, the invention

provides a method for treating diabetes in a subject receiving insulin, and one or more glucose lowering agent comprising administering a therapeutically effective amount of an EGF receptor ligand and a gastrin compound. In particular embodiments, the glucose lowering agents are a biguanide compound, a thiazolidinedione, or an α-glucosidase inhibitor, preferably metformin and a thiazolidinedione. In particular aspects, the EGF receptor ligand is Asn^{51}-hEGF51 and the gastrin compound is a gastrin-17(leu) [SEQ ID NO.14].

The invention provides methods for treating cells, preferably cells in culture using at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or compositions, or conjugates of the invention. The invention also provides cell based treatment methods using at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or compositions, or conjugates of the invention. See PCT/CA03/33595 for a description of general culture and cell based treatment methods.

In an aspect, the invention relates to a method for expanding and differentiating stem cells or progenitor cells into insulin secreting cells comprising contacting the stem cells or progenitor cells with at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention in sufficient amounts to expand and differentiate stem cells or progenitor cells. The amount of expansion and differentiation may be significantly different compared with that achieved in the absence of the compounds, composition or conjugate, in particular the amount may be significantly greater compared with an amount achieved with the compounds alone. In an embodiment, the stem cells or progenitor cells are contacted with the compounds, composition, or conjugate in culture. In another embodiment, the stem cells or progenitor cells are contacted with the compounds, composition, or conjugate in a subject. The compounds, composition or conjugate may be administered to a subject before, during, or after implantation of stem cells in the subject to expand and differentiate the stem cells in the subject. The stem cells may be obtained from pancreatic islets, umbilical cords, embryos, or stem cell lines. The method may additionally comprise administering an immunosuppressive agent.

The invention also relates to a method for enhancing proliferation of insulin secreting cells in culture comprising contacting the cells with at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, composition or conjugate of the invention in sufficient amounts to enhance proliferation of the cells. The amount of proliferation may be significantly different compared with that achieved in the absence of the compounds, composition or conjugate. In an embodiment, the amount of proliferation is significantly greater compared with the compounds alone

The invention further relates to a method for sustaining islet cells or precursor cells in culture comprising culturing the cells in the presence of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, composition, or conjugate of the invention in an amount sufficient to sustain the cells in culture. The cells may be sustained in culture for a significantly longer period of time compared with cells cultured in the absence of the compounds, composition or conjugate, or in the presence of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound. Culturing cells in the presence of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention will be particularly useful in preparing and maintaining cells intended for transplantation.

In an aspect, the invention provides a method of treating a condition and/or disease comprising administering at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention with a plurality of cells to a subject in need thereof to thereby produce a beneficial effect, preferably a sustained beneficial effect.

A method for treating a subject with a condition and/or disease described herein comprises contacting *ex vivo* a plurality of cells with at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention of the invention, optionally culturing the cells, and administering the cells to the subject in need thereof.

In embodiments of the aforementioned cell based therapeutic methods the cells are pancreatic ductal cells and the amount of compounds/composition/conjugate used in the method is generally effective to increase the amount of insulin secreting cells in the subject. The cells may be autologous (i.e. from the same subject), or may be from another individual of the same species, or from a different species.

The invention also contemplates a method for treating diabetes in a subject comprising transplanting a pancreatic islet preparation into the subject and administering a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a composition or conjugate of the invention.

In the cell based methods of the invention the number of cells administered to an individual afflicted with a condition and/or disease will vary according to the severity of the condition and/or disease, the mode of administration, and/or the site of administration. Generally a therapeutically effective amount of cells is a safe and effective amount, and in particular an amount necessary to provide one or more beneficial effect, in particular a sustained beneficial effect, or a synergistic effect.

Cells can be administered to subjects using a variety of means apparent to those of skill in the art. Suitable methods include injection of the cells into a target site in a subject. Cells may be inserted into a delivery device to facilitate injection or implantation into the subjects. Examples of delivery devices include tubes, e.g., catheters, for injecting cells and fluids into the body of a subject. Cells can be prepared for delivery in a variety of different forms. For example, the cells may be suspended in a solution or gel, or mixed with a pharmaceutically acceptable carrier, excipient, or diluent in which the cells remain viable. Pharmaceutically acceptable carriers, excipients, and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is generally sterile, and will often be isotonic. A solution of cells is preferably selected that is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms through the use of, for example, parabens, chlorobutanol, phenol, scorbic acid, thimerosal, and the like.

Modes of administration of cells include without limitation systemic intracardiac, intracoronary, intravenous, intradermal, or intra-arterial injection and injection directly into the tissue or organ at the intended site of activity, or in proximity to the site of activity. A cell preparation can be administered by any convenient route, for example by infusion or bolus injection and can be administered together with other biologically active agents. Administration in some aspects is preferably systemic. A cell preparation can be administered by any convenient route, for example by infusion or bolus injection and can be administered together with other biologically active agents.

Methods of the invention may further comprise measuring or monitoring one or more of the following markers: blood glucose, serum glucose, blood glycosylated haemoglobin, pancreatic beta cell mass, serum insulin, pancreatic insulin levels, morphometrically determined beta cell mass, C-peptide, amount of insulin secreting cells, and glucose responsiveness of insulin secreting cells.

The invention also contemplates the use of a composition comprising a combination of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound for the preparation of a medicament providing beneficial effects, preferably sustained beneficial effects in treating a condition and/or disease. In an aspect, the invention relates to the use of a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound for preparation of a medicament for providing beneficial effects, preferably sustained beneficial effects, in treating a condition and/or disease. In an embodiment the invention provides the use of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound for the preparation of a medicament for increasing (preferably sustained increase) the number and/or size of beta cells in a subject after treatment. In another embodiment the invention provides the use of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound for the preparation of a medicament for stimulation (preferably sustained stimulation) of beta cell proliferation after treatment. In a still further embodiment the invention provides the use of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound for the preparation of a medicament for treatment of Type I or Type II diabetes.

The invention additionally provides uses of a pharmaceutical composition and a conjugate of the invention in the preparation of medicaments for beneficial effects, preferably sustained beneficial effects, in the treatment of conditions and/or diseases.

Therapeutic efficacy and toxicity of compounds, compositions and conjugates of the invention may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals such as by calculating a statistical parameter such as the ED_{50} (the dose that is therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The therapeutic index is the dose ratio of therapeutic to toxic effects and it can be expressed as the ED_{50}/LD_{50} ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred.

The compounds, compositions, medicaments, and conjugates of the present invention can be administered by any means that produce contact of the active agent(s) with the agent's sites of action in the body of a subject or patient. The active ingredients can be administered simultaneously or sequentially, and in any order at different points in time, to provide the desired beneficial effects. The compounds, conjugates and compositions can be formulated for sustained release, for delivery locally or systemically. It lies within the capability of a skilled physician or veterinarian to select a form and route of administration that optimizes the effects of the compositions, conjugates, and treatments of the present invention.

In accordance with aspects of the present invention, the factors may be administered in any effective order or time interval. However, the factors are preferably "concurrently administered," meaning that independent of the order in which the factors are administered, the factors are administered within a time interval such that the effect of the factors is at least greater than additive. The factors may also be administered together.

In accordance with the present invention, each factor may be independently administered any effective number of times, including more than once, as may be indicated by a physician or veterinarian.

The compositions may be administered in oral dosage forms such as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. They may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular forms, all utilizing dosage forms well known to those of ordinary skill in the pharmaceutical arts. The compositions of the invention may be administered by intranasal route via topical use of suitable intranasal vehicles, or via a transdermal route, for example using conventional transdermal skin patches. A dosage protocol for administration using a transdermal delivery system may be continuous rather than intermittent throughout the dosage regimen.

A particular route of administration is parenteral administration, preferably peripheral parenteral administration. Parenteral administration is generally understood to refer to the injection of a dosage form into the body by a sterile syringe or some other mechanical device such as an infusion pump. For the purpose of the present invention parenteral routes include intravenous, intramuscular, subcutaneous, and intraperitoneal routes of administration. For parenteral administration, the compounds or conjugates described herein may be combined with distilled water at an appropriate pH.

The present invention includes combination treatments providing additive or synergistic activity, delivering an additive or synergistically effective amount, or an amount to provide a therapeutically effective amount of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, or a conjugate or composition of the invention. Therefore, pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in a synergistically effective amount or a therapeutically effective amount.

The dosage regimen of the invention will vary depending upon known factors such as the pharmacodynamic characteristics of the agents and their mode and route of administration; the species, age, sex, health, medical condition, and weight of the patient, the nature and extent of the symptoms, the kind of concurrent treatment, the frequency of treatment, the route of administration, the renal and hepatic function of the patient, and the desired effect. The effective amount of a drug required to prevent, counter, or arrest progression of a condition can be readily determined by an ordinarily skilled physician or veterinarian.

A composition, medicament, or treatment of the invention may comprise a unit dosage of at least one gastrin agonist, a unit dosage of at least one growth/hormone regulatory factor, and optionally a unit dosage of at least one gastrin compound.

In an aspect, a pharmaceutical composition is provided comprising a therapeutically effective suboptimal dosage of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, that are more effective at decreasing or reducing glucose levels for a sustained period following treatment compared with a dosage of any of the compounds alone.

In another aspect, an improved pharmaceutical composition is provided comprising therapeutically effective suboptimal amounts of at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound in a form for chronic or acute therapy of a condition and/or disease, in particular diabetes.

In an embodiment, the composition comprises at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound in doses that are equal to or at least 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold lower than the doses of each compound required to provide beneficial effects, preferably sustained beneficial effects, to treat a condition and/or disease.

In an aspect the invention provides a pharmaceutical composition comprising between about 0.5 to 6000, 100-1500, 100-6000, 1000-6000, 2000-6000, and 3000-6000 micrograms of a growth/hormone regulatory factor per single unit, between about 0.5 to 6000, 100-1500, 100-6000, 1000-6000, 2000-6000, and 3000-6000 micrograms of a gastrin agonist per single unit, and optionally 0.5 to 6000, 100-3000, 100-6000, 1000-6000, 2000-6000, and 3000-6000 micrograms gastrin compound per single unit.

In another aspect the invention provides a pharmaceutical composition comprising between about 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 60 micrograms/kg/day of growth/hormone regulatory factor; between about 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50; 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 60 micrograms/kg/day of a gastrin agonist, and optionally about 0.1 to 60 micrograms/kg/day of a gastrin compound.

A composition or formulation of the invention may be administered to a subject continuously for 2 weeks to 12 months, 2 weeks to 6 months, 2-16 weeks, 2 weeks to 12 weeks, and/or 2-8 weeks, or periodically.

In an embodiment, the ratio of gastrin agonist to growth/hormone regulatory factor in a composition of the invention is selected to augment the activity of the gastrin agonist and/or growth/hormone regulatory factor and to provide beneficial effects, preferably sustained beneficial effects.

A gastrin agonist and a growth/hormone regulatory factor may be in a ratio selected to augment the activity of one or both compounds to produce beneficial effects, in particular a sustained beneficial effect, and/or to produce an additive or synergistic effect. In embodiments, the ratio of a growth/hormone regulatory factor to a gastrin agonist may be from about 1:1 to 1:200, 1:1 to 1:150, 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, 1:1 to 1:5, and 1:1. In other particular embodiments, the ratio of a gastrin agonist to a growth/hormone regulatory factor may be from about 1:1 to 1:200, 1:1 to 1:150, 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, and 1:1 to 1:5.

A growth/hormone regulatory factor may be used in combination with a gastrin agonist at therapeutically effective weight ratios of between about 1:1 to 1:150, in particular 1:1 to 1:50. In another embodiment, a gastrin agonist may be used in combination with a growth/hormone regulatory factor at therapeutically effective weight ratios of between about 1:1 to 1:150, in particular 1:1 to 1:50.

The compositions of the present invention or fractions thereof typically comprise suitable pharmaceutical diluents, excipients, vehicles, or carriers selected based on the intended form of administration, and consistent with conventional pharmaceutical practices. The carriers, vehicles etc. may be adapted to provide an additive, synergistically effective or therapeutically effective amount of the active compounds.

Suitable pharmaceutical diluents, excipients, vehicles, and carriers are described in the standard text, Remington, The Science and Practice of Pharmacy, 21st Edition, 2005, University of the Sciences in Philadelphia (USIP). By way of example, for oral administration in the form of a capsule or tablet, the active components can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as lactose, starch, sucrose,

methyl cellulose, magnesium stearate, glucose, calcium, sulfate, dicalcium phosphate, mannitol, sorbital, and the like. For oral administration in a liquid form, the drug components may be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Suitable binders (e.g. gelatin, starch, corn sweeteners, natural sugars including glucose; natural and synthetic gums, and waxes), lubricants (e.g. sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, and sodium chloride), disintegrating agents (e.g. starch, methyl cellulose, agar, bentonite, and xanthan gum), flavoring agents, and coloring agents may also be combined in the compositions or components thereof.

In an aspect of the invention a pharmaceutical composition has a pH from about 7 to 10.

Formulations for parenteral administration of a composition of the invention may include aqueous solutions, syrups, aqueous or oil suspensions and emulsions with edible oil such as cottonseed oil, coconut oil or peanut oil. Dispersing or suspending agents that can be used for aqueous suspensions include synthetic or natural gums, such as tragacanth, alginate, acacia, dextran, sodium carboxymethylcellulose, gelatin, methylcellulose, and polyvinylpyrrolidone.

Compositions for parenteral administration may include sterile aqueous or non-aqueous solvents, such as water, isotonic saline, isotonic glucose solution, buffer solution, or other solvents conveniently used for parenteral administration of therapeutically active agents. A composition intended for parenteral administration may also include conventional additives such as stabilizers, buffers, or preservatives, e.g. antioxidants such as methylhydroxybenzoate or similar additives.

In an embodiment, a solid form pharmaceutical composition is provided (e.g. tablets, capsules, powdered, or pulverized form) comprising a crystalline or amorphous growth/hormone regulatory factor, gastrin agonist, and optionally a gastrin compound.

In another embodiment, the invention relates to a liquid drug formulation comprising pharmaceutically acceptable salts of a gastrin agonist, a growth/hormone regulatory factor, and optionally a gastrin compound, and to lyophilized drug formulations that can be reconstituted to provide suspensions that are stable and suitable for parenteral administration.

In a particular embodiment, the invention relates to an aqueous composition comprising pharmaceutically acceptable salts of a gastrin agonist, a growth/hormone regulatory factor, and optionally a gastrin compound, and a solvent system which effects solubilization. The invention also provides a drug comprising an aqueous formulation of pharmaceutically acceptable salts of a gastrin agonist, a growth/hormone regulatory factor, and optionally a gastrin compound with at least one solubilizer.

The compounds, conjugates, and compositions of the present invention may be sterilized by, for example, filtration through a bacteria retaining filter, addition of sterilizing agents to the composition, irradiation of the composition, or heating the composition. Alternatively, the compounds, conjugates, and compositions of the present invention may be provided as sterile solid preparations e.g. lyophilized powder, which are readily dissolved in sterile solvent immediately prior to use.

In addition to the formulations described herein, the compositions can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the fractions may be formulated with

suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil), or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions of the invention and components thereof may comprise soluble polymers as targetable drug carriers.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labelled for treatment of an indicated condition. For administration of a composition of the invention, such labelling would include amount, frequency, and method of administration.

Since the present invention relates to a method of treatment comprising a combination of active agents which may be administered separately or as conjugates, the invention also provides a kit comprising a gastrin agonist, a growth/hormone regulatory factor, and optionally a gastrin compound, a pharmaceutical composition or conjugate of the invention in kit form. The invention also relates to a pharmaceutical kit comprising one bottle with a gastrin agonist, another bottle with a growth/hormone regulatory factor, and optionally another bottle with a gastrin compound in one box. A kit may comprise a package which houses a container which contains a conjugate or composition of the invention and also houses instructions for administering the conjugate or composition to a subject.

In aspects of the invention, a pharmaceutical pack or kit is provided comprising one or more containers filled with one or more of the ingredients of a pharmaceutical composition of the invention to provide a beneficial effect, in particular a sustained beneficial effect. Associated with such container(s) can be various written materials such as instructions for use, or a notice in the form prescribed by a governmental agency regulating the labeling, manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use, or sale for human administration.

In an aspect, the invention relates to a "kit-of-parts", for example, the components to be combined according to the present invention can be dosed independently or by use of different fixed combinations with distinguished amounts of the components, i.e. simultaneously or at different time points. The parts of the kit can then be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit. Time intervals can be selected such that the effect on the condition and/or disease in the combined use of the parts is larger than the effect that would be obtained by use of any one of the components.

In an aspect the invention provides a kit of parts comprising: (a) an amount of a gastrin agonist or a pharmaceutically acceptable salt thereof in a first unit dosage; (b) an amount of a growth/hormone regulatory factor or a pharmaceutically acceptable salt thereof in a second unit dosage; and optionally (c) an amount of a gastrin compound or a pharmaceutically acceptable salt thereof in a third unit dosage, in the form of one, two or optionally three or more separate units of the components (a) to (c).

The invention further relates to a commercial package comprising at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound, together with instructions for simultaneous, separate or sequential use.

In an aspect a commercial package comprising as active ingredients at least one gastrin agonist, at least one growth/hormone regulatory factor, and optionally at least one gastrin compound is provided in the form of two, three or more separate units of the components, together with instructions for its simultaneous, separate or

sequential use, or any combination thereof, in the delay of progression or treatment of a condition and/or disease disclosed herein.

The present invention also includes compositions, kits and methods of using the compositions and kits of the invention in combination with one or more additional therapeutic agents including without limitation immunosuppressive agents, antidiabetic agents including without limitation insulin sensitivity enhancers, glucose lowering agents, insulin secretagogues, insulin, antiobesity agents, appetite regulating drugs, antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with a condition and/or disease, in particular diabetes and obesity, anti-nausea, anti-headache medications, and general medications that treat or prevent side effects.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

Example 1

Effects of a GLP-1 or an Exendin-4 in Combination with a PPI in Acutely-Diabetic NOD Mice

Objective:

NOD mice spontaneously develop insulin-dependent diabetes as a result of autoimmune destruction of pancreatic islet ß-cells. This study will be aimed at treating diabetes in NOD mice by regenerating islet ß-cells or reducing insulin dependence using a GLP-1 or exendin-4 (e.g., Byetta™) and a PPI.

Female NOD mice ages 12-16 weeks will be treated for 18 days only, with vehicle (PBS), with a GLP-1 (300 µg/kg/day) or an exendin-4 (e.g., Byetta™) (5-10 mcg per dose administered twice daily), with a GLP-1 (300 µg/kg/day) or an exendin-4 (e.g., Byetta™) (5-10 mcg per dose administered twice daily) in combination with a PPI, or GLP-1 (300 µg/kg/day) or an exendin-4 (e.g., Byetta™) (5-10 mcg per dose administered twice daily) in combination with 3 µg/kg/day of Gastrin (G1) and a PPI. Animals will be injected for 18 days, twice daily, within 2 to 5 days after diabetes onset. The fasting blood glucose (FBG) levels are expected to be 9-15 mM at diabetes onset (normal FBG <6.0 mM). The mice will be monitored daily for urine glucose levels and weekly for FBG levels during the treatment and for an additional 6 weeks after the treatment is stopped. The pancreatic insulin levels will be determined in each group as well as histological analysis of the pancreatic tissue will be performed. Pancreatic tissues will be fixed and stained for insulin producing cells. The beta cell mass will be determined by morphometric analysis.

Example 2

Clinical trial to evaluate the safety, tolerability, pharmacokinetic profile and effects of repeated doses of a GLP-1 or an Exendin-4 (e.g., Byetta™) in combination with a PPI in patients with Type I diabetes.

Study Design

This will be a randomized 3:1 treatment to vehicle control, double-blind study. A total of 20 patients with Type I diabetes will be randomized on Day 1 of the Treatment Phase. Fifteen (15) patients will be randomized to receive active study medication and 5 patients will be randomized to receive vehicle control. Additional patients may be entered into this study to ensure that 20 patients receive at least 21 days of treatment.

This study has a 2 week Baseline period, 4 week Treatment period, and 6 month (non investigational drug treatment period). After undergoing screening procedures the potential patients will enter a 14 day Baseline Phase where baseline data will be collected. During this period and throughout the study, patients will remain on their insulin regimen and will record insulin intake and blood glucose levels daily through the use of a daily diary.

Pending successful completion of the Baseline Phase, patients will enter the Treatment Phase where they will be randomized to receive either once or twice daily sc injections of a GLP-1 or an exendin-4 (e.g., Byetta™) plus a PPI, as separate injections, or once daily injections of vehicle control (as 2 separate injections to mimic active treatment). Patients will receive once daily doses in the morning after breakfast for a period of 28 days.

On Days 1 to 3 of the Treatment Phase patients will receive study medication in the morning and will stay in the clinic throughout the day and overnight. On Day 4 patients will remain in the clinic for at least 2 hr after receiving study medication and if the treatment is deemed to be well tolerated the patients will be released. They will return to the clinic every morning for the next 3 days to receive study medication and they will remain in the clinic for at least 30 min. after each dose. In the event that patients experience unacceptable tolerability during this 30 min. period, the appropriate duration of clinic time will be decided prior to patient release. On Day 8, if tolerance is acceptable and dose is increased, patients will stay in the clinic throughout the day and overnight. On Day 9 patients will remain in the clinic for at least 4 hours after receiving study medication and if the treatment is deemed to be well tolerated the patients will be released. In the event that patients experience unacceptable tolerability during this 4 hour period, the appropriate duration of clinic time will be decided prior to patient release. Patients will return to the clinic every morning for the next 19 days to receive study medication and they will remain in the clinic for at least 30 min. after each dose.

Upon completion of treatment, all patients will continue in the Follow-up Phase for an additional 6 months. During the 6-months follow-up period, patients will be instructed to continue to record their daily insulin intake and glucose levels in a daily diary and will return for monthly clinic visits.

Study Population

Twenty (20) Type 1 diabetes patients requiring insulin therapy, male or female, ages 18 – 40 years inclusive.

Study Treatments

(i) A GLP-1 (300 μg/kg/day) or an exendin-4 (e.g., Byetta™) (5-10 mcg per dose administered twice daily)

(ii) A GLP-1 (300 μg/kg/day) or an exendin-4 (e.g., Byetta™) (5-10 mcg per dose administered twice daily) and a PPI

(iii) Vehicle Control - 0.9 % normal saline

Endpoints

- Blood glucose levels
- Basal & stimulated C-peptide levels
- Hemoglobin A_{1C} (HbA_{1c}) levels
- Insulin usage

Example 3

Clinical trial to evaluate the safety, tolerability, pharmacokinetics and clinical response of repeated doses of a GLP-1 or an exendin-4 (e.g., Byetta™) in combination with a PPI in patients with Type 2 diabetes

Study Design

The study is a randomized 2:1 treatment to vehicle control, double-blind design. A total of 30 patients with Type 2 diabetes will be randomized on Day 1 of the Treatment Phase. Twenty (20) patients will be randomized to receive active study medication and 10 patients will be randomized to receive vehicle control.

This study has a 2 week Baseline period, a 4 week Treatment period, and a 6 month Follow-up period. After undergoing screening procedures the potential patients will enter a 14 day Baseline Phase where baseline data will be collected. During the Baseline period and throughout the study, patients will remain on their current oral hypoglycemic therapy with Metformin, Thiazolidinedione or both Metformin and Thiazolidinedione and will record blood glucose levels daily through the use of a daily diary. Patients will be asked to measure capillary blood glucose measurements daily before each meal and at bedtime. Once a week, patients will perform a 7-point profile, which consists of the usual pre-meal glucose measurement plus a 2 hour post-meal sample after breakfast, lunch and dinner and the bedtime sample.

Pending successful completion of the Baseline Phase, patients will enter the Treatment Phase where they will be randomized to receive either once or twice daily sc injections of a GLP-1 or an exendin-4 (e.g., Byetta™) plus a PPI, as separate injections, or once daily injections of vehicle control (as 2 separate injections to mimic active treatment). Patients will receive once daily doses in the morning after breakfast for a period of 28 days.

On Days 1 to 3 of the Treatment Phase patients will receive study medication in the morning and will stay in the clinic throughout the day and overnight. On Day 4 patients will remain in the clinic for at least 2 hours after receiving study medication and if the treatment is deemed to be well tolerated the patients will be released. Patients will return to the clinic every morning for the next 3 days to receive study medication and will remain in the clinic for at least 30 min. after each dose. In the event that patients experience unacceptable tolerability during this 30 min. period, the appropriate duration of clinic time will be decided prior to patient release. On Day 8, if tolerance is acceptable and dose is increased, patients will stay in the clinic throughout the day and overnight. On Day 9 patients will remain in the clinic for at least 4 hours after receiving study medication and if the treatment is deemed to be well tolerated the patients will be released. In the event that patients experience unacceptable tolerability during this 4 hour period, the appropriate duration of clinic time will be decided prior to patient release. Patients will return to the clinic every morning for the remainder of the treatment period to receive study medication and they will remain in the clinic for at least 30 min. after each dose.

Upon completion of treatment, all patients will continue in the Follow-up Phase for an additional 6 months. During the 6-months Follow-up period, patients will be instructed to continue to record their daily Metformin and/or Thiazolidinedione intake and glucose levels in a daily diary and will return for monthly clinic visits.

Study Population

Thirty (30) Type II diabetes patients requiring Metformin and/or Thiazolidinedione therapy, male or female, ages 30 – 60 years inclusive.

Study Treatments

(i) A GLP-1 (300 μg/kg/day) or an exendin-4 (e.g., Byetta™) (5-10 mcg per dose administered twice daily)

(ii) A GLP-1 (300 μg/kg/day) or an exendin-4 (e.g., Byetta™) (5-10 mcg per dose administered twice daily) and a PPI

(iii) Vehicle Control - 0.9 % normal saline

Endpoints

- Beta cell function as defined by the change in insulin secretion
- Hemoglobin A1C levels

The present invention is not to be limited in scope by the specific embodiments described herein, since such embodiments are intended as but single illustrations of one aspect of the invention and any functionally equivalent embodiments are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications, patents and patent applications referred to herein, or referenced in such documents are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. The citation of any reference herein is not an admission that such reference is available as prior art to the instant invention.

Table 1

GLP-1 agonist	*Source*
DAC:GLP-1	Conjuchem
Long-lasting synthetic glucagons-like peptide	Conjuchem
Long-lasting insulinotropic peptides	Conjuchem
AC2592	Amylin Pharmaceuticals/ Restoragen
AC2993 – Exenatide, Byetta™	Amylin Pharmaceuticals
Exendin-4	Eli Lilly, Alkermes, Amylin
NN2211 - GLP-1 (Liraglutide)	Novo Nordisk
ThGLP-1	Theratechnologies
ZP10	Zealand Pharma/ Aventis
Albumin:GLP-1 fusion peptide	Human Genome Sciences
BIM 51077	Roche/Ipsen
N-terminally truncated GLP-1 derivatives & analogs (lipophilic substituent attached)	Novo Nordisk PCT/DK99/00081
Derivatives of GLP-1 analogs with a lipophilic substituent	Novo Nordisk PCT/DK99/00082 US 6,458,924
N-terminally modified GLP-1 derivatives & analogs with lipophilic substituent attached and protracted profile of action (N-terminal end has a substituent comprising an optionally substituted 5- or 6-membered ring system)	Novo Nordisk PCT/DK99/00085
Derivatives of GLP-1 analogs with a lipophilic substituent (protracted profile of action)	Novo Nordisk WO 98/08871
GLP-1 fragment as insulinotropic hormone	The General Hospital Corporation WO 87/06941
GLP-1 derivatives with insulinotropic activity	The General Hospital Corporation WO 90/11296
GLP-1 analogs exhibiting enhanced stability or an enhanced capacity to stimulate insulin production	Buckley et al. WO 91/11457
GLP-1 analogs and derivatives (stimulate the secretion or biosynthesis of insulin in poorly functioning beta cells)	Eli Lilly & Co. EP 0708179-A2
N-terminal truncated GLP-1 and analogs (promote glucose uptake by cells but do not stimulate insulin expression or secretion)	Eli Lilly & Co. EP 0699686 -A2
GLP-1 analogs or derivatives for increasing the number and/or the size of beta cells and for stimulating beta cell proliferation	Novo Nordisk US 2003/0224983
GLP-1 derivatives with a lipophilic substituent and protracted profile of action	Novo Nordisk US 6268343
Pharmaceutical formulations of GLP-1 agonists	Novo Nordisk US 20030119734 A1
GLP-1 amide, fragment, analogue or derivative	Novo Nordisk US 20030083259 A1
GLP-1 compositions having protracted action	Novo Nordisk US 20010006943 A1
GLP-1 & gastrin	Transition Therapeutics PCT/CA03/
Gastrin formulations	Transition Therapeutics PCT/CA03/
Derivatives of GLP-1 analogs with a lipophilic substituent (protracted profile of action)	Novo Nordisk WO 99/43706

GLP-1 agonist	*Source*
GLP-1 and exendin derivatives with just one lipophilic substituent attached to the C-terminal amino acid residue	Novo Nordisk WO 99/43708
Modified exendins and agonists linked to one or more polyethylene glycol polymers	Amylin Pharmaceuticals WO 00/66629
Ecarin, a procoagulant protein from Echis carinatus venom	Cohesion Technologies WO 01/04146
Modified Fragments of GLP-1, exendin 3 and exendin 4	Conjuchem, Inc. US 6,514,500
GLP-1 analogs	Novo Nordisk A/S US 6,451,974
GLP-1 analogs, derivatives and active peptides	Eli Lilly and Company 6,191,102
GLP-1 Fragments	The General Hospital Corporation 6,162,907
GLP-1 molecules associated with a divalent metal cation	Eli Lilly and Company 6,133,235 5,977,071
Buccal delivery systems with GLP-1	Theratech, Inc. 5,863,555
GLP-1 Analogs	Eli Lilly and Company 5,981,488
GLP-1 mimics	Bristol-Myers Squibb Company WO 03/033671
Long lasting GLP-1	Conjuchem, Inc. US 6,593,295 US 6,514,500 US 6,329,336
Precursor GLP-1	Genzyme Corporation WO 03/014318
GLP-1 complexes	Eli Lilly and Company 6,358,924
Modified peptides	Theratechnologies Inc. WO 02/10195
GLP-1 and related molecules	Zealand Pharma A/S WO 2004/005342

Table 2

DPP IV Inhibitors

DPP IV Inhibitor	*Source*
Siagliptin (MK-0431)	Merck
Vildagliptin	Novartis
823093	GlaxoSmithKline
PSN9301	OSI Pharma
SYR322	Takeda/PPD
Sazagliptin	Bristol-Myers Squibb
SYR619	Takeda/PPD
TA-6666	Tanabe
DPP IV Inhibitors	Alantos
PHX1149	Phenomix
SSR 162369	Sanofi-Aventis
DPP IV Inhibitors	Santhera
TRC-8XXX	Torrent
DPP IV inhibitors	WO0152825 (Novartis)
4-pyrimidone derivatives	WO05016911
hexahydrodiazepinones	WO05011581
Pyrido-2,1-A-isoquionline	WO05000848
hexahydropyridoisoquinolines	WO05000846
DPP IV inhibitors	US2005007533
DPP IV inhibitors	US2005007070
DPP IV inhibitors	US20050070535
DPP IV inhibitors	US2005007053
DPP IV inhibitors	US20050065148
DPP IV inhibitors	US20050065145
DPP IV inhibitors	US20050065144
DPP IV inhibitors	US6861440
DPP IV inhibitors	US6849622
DPP IV inhibitors	EP1506967
1H-imidazo'4,5-dipyridazines	WO04108730
DPP IV inhibitors	WO05056013
DPP IV inhibitors	EP1541148
DPP IV inhibitors	WO0162266
DPP IV inhibitors	WO05075426
DPP IV inhibitors	WO05058849
DPP IV inhibitors	WO05056541
DPP IV inhibitors	WO05049022
DPP IV inhibitors, e.g., compounds 1001-1293 and examples 1 to 124	WO02053548
DPP IV inhibitors, e.g.,compounds 1000 to 1278 and 2001 to 2159	WO02067918
DPP IV inhibitors, e.g., compounds described in the examples	WO 02066627
DPP IV inhibitors, e.g., compounds described in examples I to LXIII; see also 2(28), 2(88), 2(119) 2(136) in the table reporting ICED	WO 02068420
DPP IV inhibitors, e.g., compounds described in examples 1 to 13	WO020831128
Azolidine carbonitriles	WO05033106
DPP IV inhibitors	WO05033099
DPP IV inhibitors	WO05030751
2-cyanopyrrolidine derivatives	WO04099185

DPP IV Inhibitor	*Source*
Substituted azetidines	WO04071454
2-cyanopyrrolidine derivatives	WO04048352
DPP IV inhibitors	Wo04048352
Hemisuccinate salts	WO04033455
Succinate salts of heterocyclic DPP IV inhibitors	WO04033455
Phenacyl xanthine derivatives of DPP IV inhibitors	WO04018467
3,3,4,4-tetrafluoropyrrolidine	US 6812350
Pyrido[2,1-A] isoquinoline derivatives	EP1461337 and WO03055881
DPP IV inhibitors	US20030130181
DPP IV inhibitors	WO-A-97/40832
DPP IV inhibitors	WO-A-98/19998
DPP IV inhibitors	WO-A-03/180
DPP IV inhibitors	WO-A-03/181.
DPP IV inhibitors	George R. Lankas et al, *Diabetes* 54:2988-2994, 2005
N- (N'-substituted glycyl)-2-cyano pyrrolidines (e.g., Example 1 DPP728 and LAF237)	WO 98/19998
val-pyr, val-thiazolidide, isoleucyl- thiazolidide, isoleucyl-pyrrolidide, and fumar salts of isoleucyl-thiazolidide and isoleucyl- pyrrolidide	DE19616 486 A1
N-substituted adamantyl-amino- acetyl-2-cyano pyrrolidines	WO 00/34241
amino acid 2- cyanopyrrolidine amides (e.g., FE-999011)	WO 95/15309
DPP IV inhibitors	WO 01/72290
DPP IV inhibitors	WO01/52825
DPP IV inhibitors	W003/002553
praline boronic esters	WO 9310127
DPP IV inhibitors	WO 99/61431
sulphostin	WO 9925719
N-substituted 4- to 8-membered heterocyclic rings	WO 9938501
phosphoric compounds	WO 9946272
DPP-IV prodrugs and inhibitors of the form A-B-C where C is either a stable or unstable inhibitor of DPP-IV	WO 9967278
DPP-IV prodrugs and inhibitors of the form A-B-C where C is either a stable or unstable inhibitor of DPP-IV	WO 9967279
W (substituted glycyl)-4-cyano pyrrolidines	US 6110949
N-Peptidyl-O-aroyl hydroxylamines	WO 2004/052362 and PCT/EP2003/013963
N-Peptidyl-O-aroyl hydroxylamines	Mona Patel and Colt (Expert Opinion Investig Drugs. 2003 Apr;12(4):623-33
DPP IV inhibitors	Diabetes, Vol. 47, pp.1253-1258 (1998
Glutaminyl based DPIV inhibitors	US6946480
Valyl-thiazolidide and valyl-pyrrolidide	US6548481

Table 3

Immunosuppressive Agents

Names	Company	Nature
2-amino-1,3-propanediol derivatives	Novartis	Used for preventing or treating chronic rejection in a patient receiving an organ or tissue allo- or xeno- transplant
2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride	Yoshitomi Pharmaceutical Industries, Ltd	Immunosuppression, from accelerated lymphocyte homing
40-O-(2-hydroxyethyl)-rapamycin, SDZ-RAD, Everolimus (Certican®)	Novartis Pharmaceuticals	Sirolimus (rapamycin) derivative, used for acute kidney rejection; reduces rejection and graft vasculopathy following heart transplantation by inhibiting cell proliferation
6-(3-dimethyl-aminopropionyl) forskolin	Matsumori Akia Nippon Kayaju Co Ltd	Immunosuppressing action useful also for treating autoimmune disease
6-mercaptopurine (Purinethol®, 6-MP)	Glaxo SmithKline	Used to treat Crohn's disease, inflammatory bowel disease and for organ transplant therapy
ABX-CBL (CBL-1)	Abgenix	Mouse monoclonal AB targeted against human T-cell, B cells, NK cells and monocytes, for treatment of steroid-resistant graft vs. host diseases, potential use in treatment of inflammatory and autoimmune disorders
Alefacept (human LFA-3 IgG1 fusion protein, AMEVIVE®)	University of Utah-Dermatology Dept/BIOGEN	Knocks out causative memory T-lymphocytes; Used to treat psoriasis, a T-cell mediated inflammatory disorder
HLA-B2702 peptide (Allotrap®)	SangStat Medical	Human peptide, blocks action of NK cells and T-cell mediated toxicities, used for prevention of first kidney allograft rejection
Antisense ICAM-1 inhibitor (ISIS 2302), Enlimomab, BIRR1, Alicaforsen)	ISIS-Boehringer Ingleheim	Mouse monoclonal AB blocks white blood cell adhesion to T-cell surface molecule (ICAM-1r); treatment of kidney transplant rejection
Azathioprine (Imuran®, Azasan®)	Generic, Glaxo SmithKline, Prometheus Laboratories, aaiPharma	Treatment of rheumatoid arthritis and prevention of kidney transplant rejection, and other autoimmune or inflammatory disorders such as inflammatory bowel disease
BTI-322	MedImmune	Mouse derived monoclonal AB targeted to CD2 receptor; used for prevention of first-time kidney rejection, and treatment of resistant rejection
Cladribine (Leustatin®)	Boehringer Ingleheim	Antimetabolite and immunosuppressive agent that is relatively selective for lymphocytes; used to treat lymphoid malignancies, e.g., hairy-cell leukemia.
Cyclophosphamide (CTX, Neosar®, Cytoxan®, Procytox®)	Generic	Immunosuppressant t for treatment of arthritis and other auto-immune disorders and cancers

Names	Company	Nature
Cyclosporine (cyclosporin A, cyclosporin) (Sandimmune®, Neoral®, SangCya®)	Novartis	11 amino acid cyclic peptide; blocks helper T-cell , immunosuppressant used in organ transplant therapy and other immune diseases
Demethimmunomycin" (L-683,742: also described as 31-desmethoxy-31-hydroxy-L-683,590)	Merck & Co	Treatment of autoimmune diseases, infectious diseases and/or prevention of organ transplant rejections
Dexamethasone (Decadron, Dexone, Dexasone)	Generic	An adrenocorticoid, effective immunosuppressant in various disorders
Docosahexaenoic acid (DHA)		Immunosuppressant by that lowers the proportion of T cells expressing CD4 or CD8, blocks antigen recognition process; Taku et al., Journal of Agricultural and Food Chemistry, 2000; 48(4):1047
FTY720 (oral myriocin derivative)	Novartis Pharmaceuticals	Alters lymphocyte infiltration into grafted tissues; used for prevention of organ rejection in kidney transplants
Glatiramer acetate (co-polymer-1, Copaxone®)	Teva Pharmaceuticals	Synthetic peptide copolymer; decoy that mimics structure of myelin so immune cells bind Copaxone instead of myelin; for multiple sclerosis
Glial fibrillary acidic protein (GFAP)	CalBiochem; Synx Pharma	Possesses immunosuppressive activities in diabetic animal models; Winer et al., Nature Medicine 9: 198 (2003)
Gusperimus,(15-deoxyspergualin (Spanidin®)	Bristol Myers-Squibb	Intravenous immunosuppressant; suppresses production of cytotoxic T-cells, neutrophils and macrophages
hu1124 (anti-CD11a)	XOMA	Humanized monoclonal antibody; targets CD11a receptor on surface of T cells to selectively inhibit immune system rejection of transplanted organs
Infliximab (Remicade®)	Centocor (affiliate of Johnson and Johnson)	Monoclonal AB, binds and inactivates human TNF-alpha and ; used to treat Crohn's disease and rheumatoid arthritis
Interferon	Various companies including Serono, Biogen etc	Immunomodulatory properties
ISAtx247	Isotechnika	Used to treat autoimmune diseases such as rheumatoid arthritis and psoriasis
isotretinoin		Immunosuppressant, reduces ability of T cells to proliferate in response to immune challenge. Vergelli et al., Immunopharmacology, 1997, 31:191.
Medi-500 (T10B9)	MedImmune	Intravenous monoclonal AB that targets human T-cells; treats acute kidney rejection and graft-vs-host disease
Medi-507	MedImmune/Bio-Transplant	Intravenous humanized AB directed against CD2 T-cell; used to treat corticosteroid-resistant graft vs host disease and prevention of kidney rejection
Methotrexate (Rheumatrex®, Amethopterin, Trexall®)	Wyeth Lederle, Generic	Antimetabolite used to treat Crohn's disease, severe psoriasis, and adult rheumatoid arthritis (and as an anti-cancer drug)

Names	Company	Nature
Mitoxantrone (Novantrone®)	Immunex	Antiproliferative effect on cellular immune system including T-cells, B-cells and macrophages; used to treat hormone-refractory prostate cancer, acute myelogenous leukemia and multiple sclerosis
mycophenolate mofetil (CellCept®)	Roche	Proliferation of T and B lymphocytes by blocking the synthesis of purine nucleotides; used in organ transplant therapy and inflammatory bowel disease
OKT4A	R.W.Johnson Pharmaceutical Research Institute	Mouse monoclonal AB targeted against human CD4 T cell; used for prevention of kidney transplant rejection when used in combination with other immunosuppressant drugs
Muromonab-CD3 (Orthoclone OKT3®)	R.W.Johnson Pharmaceutical Research Institute	Monoclonal AB that binds to receptor sites on T-cells, preventing activation by transplanted tissue
Prednisolone (Deltasone®, Oraone®)		Corticosteroid, suppresses inflammation associated with transplant rejection
basiliximab (Simulect®)	Novartis Pharmaceuticals	Monoclonal AB that binds to receptor sites on T-cells, preventing activation by transplanted tissue (renal transplant)
S100β	glial protein	Possesses immunosuppressive activities in diabetic animal models
Sirolimus, Rapamycin (Rapamune®)	Wyeth-Ayerst Laboratories	Immunosuppressant and potent inhibitor of cytokine (e.g.IL-2)-dependent T-cell proliferation (kidney transplant)
Tacrolimus (Prograf; FK-506)	Fujisawa	Interferes with IL-2 TCR communication
Antithymocyte immunoglobulin (ATGAM, Thymoglobulin®)	SangStat Medical Corporation, Pharmacia and Upjohn	Anti-human thymocyte immunoglobulin; used in reversal of acute kidney transplant rejection and will likely be used off-label for transplant induction therapy
efalizumab (Xanelim®)	XOMA	T-cell modulator that target T-cells through interactions with adhesion molecules on endothelial cell surface, target migration of T-cells into the skin and target activation of T-cells; Used to treat Psoriasis
Daclizumab (Zenapax®), HAT (Humanized Anti-Tac), SMART anti-Tac, anti-CD25, and humanized anti-IL2-receptor	Protein Design Laboratories/Roche	Monoclonal AB inhibits binding of IL-2 to IL-2 receptor by binding to IL-2 receptor; suppresses T cell activity against allografts (renal transplant)

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising thereapeutically effective amounts at least one gastrin agonist and at least one growth/hormone regulatory factor(s), and optionally a gastrin compound that provides beneficial effects relative to each compound alone, and optionally a pharmaceutically acceptable carrier, excipient, or vehicle.

2. A pharmaceutical composition according to claim 1 for treatment of diabetes in a form that provides normal or reduced blood glucose levels in a subject that persist for a prolonged period of time after administration.

3. A pharmaceutical composition according to claim 1 or 2 wherein the gastrin agonist(s) and growth/hormone regulatory factor(s) are present in doses that are at least about 1.1 to 1.4, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold lower than the doses of each compound alone required to treat a condition and/or disease.

4. A pharmaceutical composition according to claim 1 or 2 comprising an additive amount of at least one gastrin agonist and at least one growth/hormone regulatory factor in a pharmaceutically acceptable excipient, carrier, or vehicle.

5. A pharmaceutical composition according to claim 1 or 2 comprising a synergistically effective amount of at least one gastrin agonist and at least one growth/hormone regulatory factor in a pharmaceutically acceptable excipient, carrier, or vehicle.

6. A pharmaceutical composition according to any preceding claim wherein the beneficial effects are reduced insulin dependency or delivery.

7. A pharmaceutical composition as claimed in claim 6 wherein the insulin dependency or delivery is reduced by 35-75%.

8. A pharmaceutical composition according to any preceding claim wherein the beneficial effects are sustained beneficial effects that persist for a prolonged period of time after termination of treatment.

9. A pharmaceutical composition as claimed in claim 8 wherein the beneficial effects are sustained for at least about 2, 4, 5, 6, or 10 weeks, 2 to 4 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment.

10. A pharmaceutical composition according to any preceding claim wherein the therapeutically effective amounts are sufficient to provide at least about a 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, or 50% increase in pancreatic insulin levels.

11. A pharmaceutical composition according to any preceding claim wherein the therapeutically effective amounts are sufficient to provide at least about a 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% decrease in blood glucose levels.

12. A pharmaceutical composition according to any preceding claim wherein the therapeutically effective amounts are sufficient to provide about normal blood glucose levels for a period of at least 2, 4, 6, 8, or 10 weeks, 2 to 4 weeks, 2 to 6 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment.

13. A pharmaceutical composition according to any preceding claim wherein the growth/hormone regulatory factor is a GLP-1 agonist preferably GLP-1(1-37), GLP-1(7-36) amide, fragments, analogues, and derivatives thereof, and active metabolites and prodrugs of GLP-1.

14. A pharmaceutical composition according to claim 13 wherein the GLP-1 agonist is a GLP-1 agonist listed in Table 1.

15. A pharmaceutical composition according to claim 13 wherein the GLP-1 agonist is $Arg^{34}Lys^{26}$(Ne(γ-Glu(Na-hexadecanoyl)))-GLP-1(7-37).

16. A pharmaceutical composition according to claim 13 wherein the GLP-1 agonist is exenatide.

17. A pharmaceutical composition according to any preceding claim wherein the gastrin agonist is a proton pump inhibitor.

18. A pharmaceutical composition according to claim 17 wherein the proton pump inhibitor is leminoprazole, nepaprazole, tenatoprazole, omeprazole, esomeprazole, lansoprazole, rabeprazole, pantoprazole, pariprazole, (−)pantoprazole, soraprazan, ilaprazole, AZD-0865, hydroxyomeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereo.

19. A pharmaceutical composition according to any preceding claim comprising a gastrin compound selected from the group consisting of gastrin 71 [SEQ ID NO. 5, residues 22-92], gastrin 52 [SEQ ID NO. 6], gastrin 34 (big gastrin) [SEQ ID NO. 1 or 2], gastrin 17 (little gastrin) [SEQ ID NO. 3 or 4], gastrin 14 [SEQ ID NO. 7], gastrin 8, gastrin 6 [SEQ ID NO. 8 or 9], pentagastrin, and tetragastrin.

20. A pharmaceutical composition according to any preceding claim comprising 15Leu gastrin 17 [SEQ ID NO. 4]

21. A pharmaceutical composition according to claim 19 or 20 wherein the proton pump inhibitor is one or more of omeprazole, hydroxyomeprazole, esomeprazole, tenatoprazole, lansoprazole, pantoprazole, rabeprazole, dontoprazole, habeprazole, periprazole, ransoprazole, pariprazole, leminoprazole; or a free base, free acid, salt, hydrate, ester, amide, enantiomer, isomer, tautomer, polymorph, metabolite or prodrug thereto.

22. A method for treating or preventing a condition and/or disease in a subject comprising administering to the subject a therapeutically effective amount of a composition according to any preceding claim, to produce a sustained beneficial effect.

23. A method of treatment comprising administering to a subject a therapeutically effective amount of at least one growth/hormone regulatory factor in combination with administration of at least one gastrin agonist, and optionally at least one gastrin compound, which upon administration to a subject with symptoms of diabetes provides sustained beneficial effects.

24. A method according to claim claim 22 or 23 wherein therapeutically effective amounts of the gastrin agonist, growth/hormone regulatory factor and optionally gastrin compound are combined prior to administration to the subject.

25. A method as claimed in claim 24 wherein therapeutically effective amounts of the gastrin agonist and growth/hormone regulatory factor are administered to the subject sequentially.

26. A method of treating diabetes comprising administering a gastrin agonist, a growth/hormone regulatory factor and optionally a gastrin composition or a composition of any preceding claim with a plurality of stem cells or progenitor cells to a subject in need thereof to thereby produce beneficial effects, preferably sustained beneficial effects.

27. A method according to any preceding claim wherein the condition and/or disease is diabetes.

28. A method for inducing islet neogenesis in a subject comprising contacting islet precursor cells with at least one gastrin agonist and at least one growth/hormone regulatory factor(s), and optionally at least one gastrin compound, in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

29. A method for expanding and differentiating stem cells into insulin secreting cells comprising contacting the stem cells with an effective amount of at least one gastrin agonist and at least one growth/hormone regulatory factor(s), and optionally at least one gastrin compound or a composition according to any preceding claim.

30. A method for treating diabetes in a subject receiving insulin and one or more glucose lowering agent comprising administering a therapeutically effective amount of an EGF receptor ligand and a gastrin compound.

31. A method according to claim 30 wherein the glucose lowering agents are a biguanide compound, a thiazolidinedione, or an α-glucosidase inhibitor.

32. A method according to claim 31 wherein the glucose lowering agents are metformin and a thiazolidinedione.

33. A method according to any one of claims 30 to 32 wherein the EGF receptor ligand is Asn^{51}-hEGF51.

34. A method according to any one of claims 30 to 33 wherein the gastrin compound is a gastrin-17(leu) [SEQ ID NO. 4].

35. Use of a composition comprising a combination of at least one gastrin agonist and at least one growth/hormone regulatory factor(s), and optionally at least one gastrin compound or a composition of any preceding claim for the preparation of a medicament for the treatment of diabetes.

SEQUENCE LISTINGS

SEQ ID NO. 1
Gastrin 34

Xaa-Leu-Gly-Pro-Gln-Gly-Pro-Pro-His-Leu-Val-Ala-Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe

Xaa = pyroglutamate, Gln or no amino acid residue

SEQ ID NO. 2
Gastrin 34

Xaa-Leu-Gly-Pro-Gln-Gly-Pro-Pro-His-Leu-Val-Ala-Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Leu-Asp-Phe

Xaa = pyroglutamate, Gln or no amino acid residue

SEQ ID NO. 3
Gastrin 17

Xaa-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe

Xaa = pyroglutamate or no amino acid residue

SEQ ID NO. 4
Gastrin 17

Xaa-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Leu-Asp-Phe-amide

Xaa = pyroglutamate or no amino acid residue

SEQ ID NO. 5
Gastrin preprotein
Gastrin 71 (residues 22 to 92)

mqrlcvyvli falalaafse aswkprsqqp daplgtganr dlelpwleqq gpashhrrql gpqgpphlva dpskkqgpwl eeeeeaygwm dfgrrsaede n

SEQ ID NO. 6
Gastrin 52

DLELPWLEQQ GPASHHRRQL GPQGPPHLVA DPSKKQGPWL EEEEEAYGWM DF

Asp-Leu-Glu-Leu-Pro-Trp-Leu-Glu-Gln-Gln--Gly-Pro-Ala-Ser-His-His-Arg-Arg-Gln-Leu--Gly-Pro-Gln-Gly-Pro-Pro-His-Leu-Val-Ala--Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-Leu--Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe

SEQ ID NO. 7
Gastrin 14

WLEEEEEAYGWM DF

Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe

SEQ ID NO. 8
Gastrin 6

YGWM DF

SEQ ID NO. 9
Gastrin 6

YGWL DF

SEQ ID NO. 10

Tyr-Gly-Trp-Met-Asp-Phe

SEQ ID NO. 11

Tyr-Gly-Trp-Leu-Asp-Phe

SEQ ID NO. 12

Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala

SEQ ID NO. 13

TrpMetAspPhe-Xaa

Xaa = NH_2

SEQ ID NO. 14

TrpLeuAspPhe-Xaa

Xaa = NH_2

SEQ ID NO. 15
GLP-1 (1-37)

His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly

SEQ ID NO. 16
GLP-1 (1-36)

His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg

SEQ ID NO. 17
GLP-1 (1-36)NH_2

His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Xaa

Xaa = NH_2

SEQ ID NO. 18
GLP-1 (7-37)

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly

SEQ ID NO. 19
GLP-1 (7-36)

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg

SEQ ID NO. 20
GLP-1 (7-36)amide

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Xaa

Xaa = NH_2

SEQ ID NO. 21
Exendin

HX1X2GTFITSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS

HisX1X2Gly-Thr-Phe-Ile-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser

X1X2 = SD or GE

SEQ ID NO. 22
Exendin-3 (Heloderma horridum horridum) Genbank Accession No. P20394

hsdgtftsdl skqmeeeavr lfiewlkngg pssgappps

His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Asp-Leu--Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg--Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser

SEQ ID NO. 23
Exendin-4 (Heloderma suspectum) Genbank Accession No. HWGH4G

hgegtftsdl skqmeeeavr lfiewlkngg pssgappps

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu--Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg--Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser

SEQ ID NO. 24
Exendin-4 amide

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-Xaa

Xaa = NH_2

SEQ ID NO. 25
Exendin (1-30)

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly

SEQ ID NO. 26
Exendin-4 (1-30) amide

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-Xaa

Xaa = NH_2

SEQ ID NO. 27
Exendin

HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGX

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-X

X = P or Y

SEQ ID NO. 28
Exendin 4(1-31)

HGEGTFTSDLSKQMEEAVR LFIEWLKNGGPY

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Ala-Val-Arg--Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Tyr

SEQ ID NO. 29
Exendin-4(9-39)

DLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS

Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser

SEQ ID NO. 30
Exendin

HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPSKKKKKK

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-Lys-Lys-Lys-Lys-Lys-Lys

SEQ ID NO. 31
Exendin-4 (1-28) amide

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-Xaa

Xaa = NH_2

SEQ ID NO. 32
^{14}Leu, ^{25}Phe exendin-4 amide

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-Xaa

Xaa = NH_2

SEQ ID NO. 33
^{14}Leu, ^{25}Phe exendin-4 (1-28) amide

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-Xaa

Xaa = NH_2

SEQ ID NO. 34
^{14}Leu, ^{22}Ala, ^{25}Phe exendin-4 (1-28) amide

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Ala Ile Glu Phe Leu Lys Asn-Xaa

Xaa = NH_2

SEQ ID NO. 35
Exendin-4 precursor
P26349 and AAB51130, 87 aa

mkiilwlcvf glflatlfpi swqmpvesgl ssedsasses faskikrhge gtftsdlskq meeeavrlfi ewlknggpss gapppsg

SEQ ID NO. 36
PRT Human EGF (epidermal growth factor)
UniProtKB/Swiss-Prot P01133

Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His
Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn
Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys
Trp Trp Glu Leu Arg

SEQ ID NO. 37
PRT Recombinant human EGF51N

Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His
Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn
Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Ser Leu Lys
Tyr Tyr Asn

SEQ ID NO. 38
PRT Recombinant human EGF51A

Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His
Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn
Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys
Trp Trp Ala

SEQ ID NO. 39
PRT Recombinant human EGF51Q

Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His
Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn
Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys
Trp Trp Gln

SEQ ID NO. 40
PRT Recombinant human EGF51S

Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His
Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn
Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys
Trp Trp Ser

SEQ ID NO. 41
DNA Artificial Sequence DNA encoding EGF51N

aactctgact ccgaatgtcc attgtctcac gacggttact gtttgcacga cggtgtttgt
atgtacatcg aagctttgga caagtacgct tgtaactgtg ttgtcggtta catcggtgaa
agatgtcaat acagagactt gaagtggtgg aattgagata a

SEQ ID NO. 42
Homo sapiens TGF

Val Val Ser His Phe Asn Asp Cys Pro Asp Ser His Thr Gln Phe Cys
Phe His Gly Thr Cys Arg Phe Leu Val Gln Glu Asp Lys Pro Ala Cys

Val Cys His Ser Gly Tyr Val Gly Ala Arg Cys Glu His Ala Asp Leu
Leu Ala

SEQ ID NO. 43
Homo sapiens TGF-α

GenBank Accession No.NP_003227

mvpsagqlal falgivlaac qalenstspl sadppvaaav vshfndcpds htqfcfhgtc
rflvqedkpa cvchsgyvga rcehadllav vaasqkkqai talvvvsiva lavliitcvl
ihccqvrkhc ewcralicrh ekpsallkgr tacchsetvv

Met-Val-Pro-Ser-Ala-Gly-Gln-Leu-Ala-Leu--Phe-Ala-Leu-Gly-Ile-Val-Leu-
Ala-Ala-Cys-Gln-Ala-Leu-Glu-Asn-Ser-Thr-Ser-Pro-Leu-Ser-Ala-Asp-Pro-Pro-
Val-Ala-Ala-Ala-Val-Val-Ser-His-Phe-Asn-Asp-Cys-Pro-Asp-Ser-His-Thr-Gln-
Phe-Cys-Phe-His-Gly-Thr-Cys-Asn-Arg-Phe-Leu-Val-Gln-Glu-Asp-Lys-Pro-Ala-
-Cys-Val-Cys-His-Ser-Gly-Tyr-Val-Gly-Ala-Arg-Cys-Glu-His-Ala-Asp-Leu-
Leu-Ala-Val--Val-Ala-Ala-Ser-Gln-Lys-Lys-Gln-Ala-Ile-Thr-Ala-Leu-Val-
Val-Val-Ser-Ile-Val-Ala-Leu-Ala-Val-Leu-Ile-Ile-Thr-Cys-Val-Leu-Asn-Ile-
His-Cys-Cys-Gln-Val-Arg-Lys-His-Cys-Glu-Trp-Cys-Arg-Ala-Leu-Ile-Cys-Arg-
His-Glu-Lys-Pro-Ser-Ala-Leu-Leu-Lys-Gly-Arg-Thr-Ala-Cys-Cys-His-Ser-Glu-
Thr-Val-Val-Asn

SEQ ID NO. 44

Amylin
Homo sapiens

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn Val
Gly Ser Asn Thr Tyr

SEQ ID NO. 45

Amylin
Homo sapiens
GenBank Accession No. NP_000406

mgilklqvfl ivlsvalnhl katpieshqv ekrkcntatc atqrlanflv hssnnfgail
sstnvgsnty gkrnavevlk replnylpl

Met-Gly-Ile-Leu-Lys-Leu-Gln-Val-Phe-Leu-Ile-Val-Leu-Ser-Val-Ala-Leu-Asn-
His-Leu-Lys-Ala-Thr-Pro-Ile-Glu-Ser-His-Gln-Val-Glu-Lys-Arg-Lys-Cys-Asn-
Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-
Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr-Gly-Lys-
Arg-Asn-Ala-Val-Glu-Val-Leu-Lys-Arg-Glu-Pro-Leu-Asn-Tyr-Leu-Pro-Leu

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001976

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 38/26*** (2006.01), ***A61K 31/4439*** (2006.01), ***A61K 38/18*** (2006.01), ***A61P 3/10*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K 38/26 (2006.01), A61K 31/4439 (2006.01), A61K 38/18 (2006.01), A61P 3/10 (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Databases: Delphion, PubMed, Scopus.
Keywords: GLP-1, glucagon like peptide, exendin, exenatide, gastrin, proton pump inhibitor, *prazole, epidermal growth factor, diabetes

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2005072045 A2 (WARATAH PHARMACEUTICALS, INC.), 11 August 2005 see page 9, line 38 to page 13, line 2; page 16, line 27 to page 17, line 33	1 - 29 and 35
X	VON HERRATH, M. E1-INT (Transition Therapeutics/Novo Nordisk), Curr Opin Investig Drugs, October 2005 vol. 6, pages 1037-1042 ISSN 1472 - 4472 see whole document	30 - 34
P, X	WO2006000567 A2 (NOVO NORDISK A/S) 5 January 2006	1 - 29 and 35

[ ] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2007 (14 - 02 - 2007)	3 April 2007 (03-04-2007)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Antonio Candeliere 819- 934-7935

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2006/001976

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2005072045	11-08-2005	AU2005207870 A1	11-08-2005
		CA2554458 A1	11-08-2005
		EP1711532 A2	18-10-2006
WO2006000567	05-01-2006	NONE	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001976

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 22 - 34

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 22 - 34 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. Regardless, this Authority has carried out a search based on the alleged effects or uses of the products defined in claims 22 - 34.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

The claims are directed to a plurality of inventive concepts as follows:

(see extra sheet)

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Continuation of Box III

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1 - 29 and 35 are directed to a pharmaceutical composition, and, its use for treating diabetes, for inducing islet neogenesis, and for expanding and differentiating stem cells into insulin secreting cells; said composition comprising at least one gastrin agonist and a growth hormone regulatory factor(s), and optionally a gastrin compound.

Group B - Claims 30 - 34 are directed to a method for the treatment of diabetes in a subject receiving insulin and one or more glucose lowering agents, comprising administering an EGF receptor ligand and a gastrin compound.

The claims must be limited to one inventive concept as set out in Rule 13 of the PCT.

An *a posteriori* analysis has concluded that the composition, comprising at least one gastrin agonist and at least one growth hormone regulatory factor(s), and its use for the treatment of diabetes, were disclosed in WO2005072045. Therefore, there is no unifying feature linking claim groups A and B.